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INFLUENCE OF THE DIET OF BLATTIDAE ON SOME OF THEIR INTESTINAL PROTOZOA[1]

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INTRODUCTION

Many studies have been made with respect to the occurrence, cultivation, cytology, and life cycles of the intestinal protozoa of the common Blattidae, but only one investigation, that of Sassuchin (1931), is in any way concerned with the influence of the food of the host on the protozoa inhabiting its intestine. The effects of the diet of other invertebrate hosts upon their intestinal fauna have also received but little attention, except for a few interesting investigations, such as those on termites made by Cleveland (1925a and b) and Lund (1930). Data relative to the influence of food suppply on free-living protozoa are given in papers by Adolph (1929) and Harding (1937a). Similar studies on the intestinal protozoa of various vertebrate hosts are discussed in the work of Hegner (1923 and 1937), Becker (1932 and 1937), MacLennan (1934), Becker and Morehouse (1935, 1936, and 1937), and Hegner and Eskridge (1937a and b).

Since many of the intestinal protozoa of cockroaches live directly on the food eaten by the host, a study of the influence of its diet on the parasites was deemed to be of interest. Roaches are especially favorable hosts for such an investigation because they harbor a great variety of intestinal protozoa. The forms to which special attention was paid in this work were *Nyctotherus ovalis, Endamoeba blattae,*[2] *Endolimax blattae, Lophomonas striata,* and *Lophomonas blattarum.* The principal points considered were:

(1) Persistence of infection with the various parasites when the hosts were kept under the following conditions: starvation, high-carbohydrate, high-fat, and high-protein diet.

(2) A qualitative and semi-quantitative study, with the help of staining methods, on the influence of the various diets on the polysaccharide and fat reserves of the parasites.

(3) A study of the influence of the foregoing factors on the size of *N. ovalis.*

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[2] In harmony with the suggestion of Morris, the amoebae of intermediate size, corresponding to *E. thomsoni* were counted as belonging to *E. blattae.*

(4) A quantitative study of the influence of the above factors on the numbers of *N. ovalis* persisting or developing.

MATERIALS AND METHODS

The roaches were collected at several localities in Washington, D. C. In the initial studies to determine the percentage of infection, three species, *Periplantea americana* (L.), *Blatta orientalis* L., and *Blattella germanica* (L.), were examined. However, since these preliminary observations showed that *P. americana* had a higher incidence of infection than either of the others and occurred in larger numbers in this region, it was used exclusively in subsequent investigations. Other factors which made it especially favorable for these studies were its large size, its hardiness, and its ability to endure starvation for long periods.

Preliminary observations revealed no appreciable difference between the percentage of infection in full grown animals and that in the developmental stages. Consequently, both nymphs and adults were employed in the experimental work. Although nematodes were found in the intestines of many of the roaches, there was no indication that they had any influence on the protozoan population.

Persistence of Infection

For these experiments, groups of four hundred roaches were maintained under each of the following conditions:

(1) Starvation.

(2) High-carbohydrate diet, consisting of cornmeal.

(3) High-protein diet provided by "Protein Milk Powder" having an approximate composition of: (a) Protein, 37.0%; (b) Milk fat, 26.5%; (c) Lactose, 26.0%; (d) Minerals (ash), 6.0%; (e) Lactic acid, 3.0%; (f) Calcium, 1.7%; (g) Moisture, 1.5%; (h) Phosphorus, 1.0%; (i) Iron, 0.001%.

(4) High-fat diet, composed of vegetable fat.

All of the experimental animals were provided with an ample supply of water.

Every series of four hundred roaches, except in the case of the starved animals, was divided into two lots of two hundred individuals each. The members of one group were kept in batches of about forty in glass jars of one gallon capacity, thus giving an opportunity to study the infections in a population. Those of the other group were placed individually in half-pint milk bottles in order to avoid the possibility of new infections becoming established. In the starvation experiment, it was found necessary to keep all of the animals individually because they became cannibalistic when starved.

The initial infection of the hosts was determined for roaches obtained from different localities and at various seasons of the year. These determinations were made at the times when the different series were set up. In making examinations for qualitative studies, the hind-gut was removed, macerated in a drop of 0.7% saline on a glass slide, and examined with a compound microscope. The intestinal contents of forty individuals from the mass and individual cultures of the various series of test animals were examined in this way at the end of two weeks and the kinds of protozoan parasites present were noted. Other groups, of forty roaches each, were studied in the same way at the end of four-, six-, eight-, and ten-week periods.

Studies on Polysaccharide and Fat Reserves of Parasites

For the studies on the polysaccharide reserves of the parasites, permanent slides were prepared from cover-glass smears of the intestinal contents fixed with Carnoy's fixative and stained by Bauer's (1933) modification of the Feulgen technique. Controls were made by using smears of the intestinal contents of the same host and staining them after one hour's digestion with filtered saliva.

Several hundred slides were prepared in the manner outlined above and later used (1) in making observations for the presence of glycogen in the various parasites, (2) in semi-quantitative studies on the amount of paraglycogen present in *Nyctotherus ovalis,* and (3) in determining the influence of the different diets on the size of *N. ovalis.*

In making the semi-quantitative studies on *N. ovalis,* four standards having varying amounts of paraglycogen were selected as a basis for comparison. From twenty-five to one hundred individuals, depending on the number present in the slides, were observed for each diet initially, after four weeks, and again after eight weeks, in order to determine the percentage of animals that corresponded to each of the standards used.

The influence of the various diets on the size of *N. ovalis* was studied by measuring the length and width of the same animals that were used in making the paraglycogen determinations. A micrometer eye-piece was employed for these measurements.

Since is was found difficult to get the smears to adhere to cover-glasses when formalin was used as a fixative, permanent slides were not made for investigations on fat, but the routine slides were stained directly with Sudan III.

Number of N. ovalis *per Host*

In studying quantitatively the influence of the various diets on the number of persisting parasites, *N. ovalis* alone was considered, since its size and regular occurrence make it best suited for such observations. In order to have all of the animals as nearly uniform in size as possible, only adult roaches or large nymphs were used. The feeding was carried on as in the experiments described above, except that all of the specimens used were kept in individual bottles. The initial number of parasites present was determined and subsequent counts were made after periods of two and four weeks, respectively.

The preparations for counting were made by dissecting the host animals, removing the entire hind-gut, macerating it completely in a drop of 0.7% saline on a glass slide, and covering it with a rectangular cover-glass (40 × 22 mm). In most cases a total count was made, using the 16-mm objective and moving the slide by means of a mechanical stage. However, when very large numbers of parasites were encountered, a slide marked off in centimeter squares was employed; several centimeter areas were counted and the total number of parasites present was then calculated.

EXPERIMENTAL DATA

Persistence of Infection

The purpose of the first experiments was to determine how long the parasitic protozoa under consideration would survive in each of the series described above.

The complete results obtained for the mass cultures are given in Table 1, while those for the individual cultures are shown in Table 2.

The percentage of infection with *N. ovalis* remained at about the same level, regardless of starvation or the kind of food supplied. Results obtained with the mass and individual cultures were essentially identical, indicating that the maintenance of a high infection rate was not due to reinfection.

On the other hand, variations in host diet did influence, more or less markedly, the persistence of infection with the flagellates and amoebae. *Lophomonas blattarum* was entirely eliminated by starvation, as well as by a diet high in protein; it was also practically eliminated by the high-fat diet. The high-carbohydrate diet was the most favorable for this species, since the infection, although becoming lighter, persisted throughout the ten weeks of the experiment.

Lophomonas striata also disappeared from some hosts on high-fat and high-protein diets, but it was not influenced as much by starvation as was *L. blattarum*. The former parasite, like the latter, persisted longer and in a higher percentage of host animals when the diet was high in carbohyrates, but at the end of the experiment the percentage of infection for *L. striata* was higher than it was for *L. blattarum*.

TABLE 1.—*Persistence of parasites when hosts were kept in mass cultures: (a) at start; (b) 2 wks.; (c) 4 wks.; (d) 6 wks.; (e) 8 wks.; and (f) 10 wks. after isolation*

Parasite	High-carbohydrate diet % of roaches infected						High-protein diet % of roaches infected						High-fat diet % of roaches infected					
	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)
N. ovalis	62	77	77	—	60	72	76	65	58	50	55	55	76	47	60	65	62	42
L. striata	40	52	42	—	30	25	12	7	2	0	2	0	12	0	0	2	2	2
L. blattarum	35	17	7	—	25	22	7	5	0	0	0	0	7	7	2	7	2	2
Endamoeba blat. ..	50	72	45	—	45	47	37	22	5	0	5	15	37	5	10	7	25	15
Endolimax blat. ...	48	70	42	—	35	30	37	15	5	30	25	40	37	2	2	15	20	22
Small flag.	78	90	97	—	90	85	100	82	85	87	75	70	100	87	85	92	82	67

The amoebae decreased decidedly under starvation, *Endolimax blattae* being entirely eliminated after eight weeks. Again, on the high-carbohydrate diet, the infection with both species persisted throughout the experiment in a relatively large number of individuals. On the high-fat and high-protein diets, *Endamoeba blattae* showed a decrease in infection rate, but was not entirely eliminated. The results obtained with the high-fat and high-protein diets on *Endolimax blattae* were somewhat irregular. With both of these diets, the infection rate declined very definitely after two to four weeks but became relatively heavy again after four to six weeks. There are several possible explanations for these results. In the first place, the infection rate in the roaches used might not have been as uniform as was supposed. However, this does not seem very likely because similar results were obtained for two different diets with both the mass and the individual cultures. Another possibility is that the infection became very light during the first few weeks of the experiment and, as a consequence, the number of individuals present was so small that they were overlooked when the intestinal contents were examined. Those amoebae which persisted possibly represented adaptable strains which by subsequent division produced the heavy infections present later in the experiments.

Besides the five species of protozoa already discussed, a number of species of very small flagellates were frequently present in the hind-gut of *Periplaneta americana*.

TABLE 2.—*Persistence of parasites when hosts were kept in individual cultures: (a) at start; (b) 2 wks.; (c) 4 wks.; (d) 6 wks.; (e) 8 wks.; and (f) 10 wks. after isolation*

Parasite	Starvation % of roaches infected						High-carbohydrate diet % of roaches infected						High-protein diet % of roaches infected						High-fat diet % of roaches infected					
	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)	(a)	(b)	(c)	(d)	(e)	(f)
N. ovalis	62	62	55	85	72	64	70	70	57	75	75	70	62	72	57	70	65	65	62	57	70	62	70	77
L. striata	40	40	37	22	10	25	24	37	24	22	22	27	15	15	5	0	2	0	15	5	2	7	0	5
L. blattarum	35	20	5	0	0	0	28	20	24	20	15	12	7	10	0	0	0	0	7	2	10	0	0	2
Endamoeba blat.	50	42	17	5	2	4	44	55	46	55	40	22	40	42	10	17	17	15	40	15	22	7	10	17
Endolimax blat.	48	50	0	10	0	0	42	37	26	42	35	20	45	30	17	30	62	32	45	7	5	2	45	40
Small flag.	78	82	87	97	90	96	84	100	90	90	87	90	97	92	82	82	92	90	97	87	92	85	92	82

They were not studied in detail, but as can be seen from the tables, the various diets did not appreciably influence the persistence of these organisms as a whole.

In general, there was very little difference in the results obtained for the mass and individual cultures, indicating that the transfer of parasites from one host to another did not play an important rôle in the persistence of the parasites.

Carbohydrate Reserves

Carbohydrate reserves which are stored in the form of polysaccharides have been demonstrated in many protozoa, both in free-living and in parasitic forms (for a detailed list see von Brand, 1935). There are, however, to the writer's knowledge few studies in which the influence of the diet of a host animal on the carbohydrate reserves of its intestinal protozoa has been considered. In the present investigation, the glycogen and paraglycogen content of the five most common intestinal protozoa of the cockroach was studied qualitatively, while a semi-quantitative study was carried out on *N. ovalis*. Only the motile forms were studied, cysts occurring too sporadically for detailed study.

In general, it may be said that, except for *N. ovalis,* the observed species of parasites showed little, if any, storage of glycogen. *Endamoeba blattae* and *Endolimax blattae* ingested large numbers of starch grains when the host was fed on a high-carbohydrate diet; but, surprisingly, only a very little glycogen was found and that was diffused in the cytoplasm. *Lophomonas striata* has no cytostome and contains no solid food. Its food probably consists of dissolved material which is taken in by osmosis through the body surface. It ordinarily contained only traces of glycogen but the amount present increased on a high-carbohydrate diet, becoming especially abundant along the numerous rod-like structures which characterize the species. *Lophomonas blattarum* contained numerous food particles, many of which were starch grains, but the staining methods employed showed no trace of glycogen as a storage product.

N. ovalis, on the other hand, contains a very large amount of glycogenous material which is apparently paraglycogen because it is relatively insoluble in water. This reserve carbohydrate occurs in the form of granules which show considerable variation in shape and size but usually have the form of a more or less rounded disc. Between the anterior end of the body and the macronucleus, these granules occur in very large numbers forming a bean-shaped mass. Although the granules are most numerous in this anterior region, typically some are also scattered throughout the rest of the cell. As will be seen from the semi-quantitative studies, this ciliate, under certain conditions, becomes almost entirely filled with the paraglycogen granules.

As previously stated, four standards (Fig. 1) based upon four selected individuals, were established as references. The first contains only a small number of scattered paraglycogen granules; the second has the bean-shaped mass at the anterior end packed with granules and also a few scattered granules; the third has relatively large numbers of granules in the various body regions, while the fourth is entirely filled with the glycogenous granules.

Significant results (Table 3) were obtained in starved animals and in roaches given a high-carbohydrate diet. In the former, parasites with much paraglycogen had markedly decreased after four weeks, but there was no further decrease when

FIG. 1. Polysaccharide storage in *Nyctotherus ovalis*. (The granules represent the polysaccharide.) 1–4 represent individuals showing increasing amount of paraglycogen, about ×200.

starvation was continued for a longer period. The other experiment gave exactly opposite results, the ciliates having but little reserve carbohydrate showing a decrease, while those in which the paraglycogen content was high, increased. In this instance also, striking results were obtained after four weeks, but a continued feeding of the same diet produced little further change. In both cases, therefore, the maximal changes had occurred within four weeks.

The two remaining experiments gave less significant results. With the high-protein diet, there was a slight increase in the carbohydrate reserve after four weeks, but at the end of eight weeks, the paraglycogen of the parasites dropped again to the amount originally present. On the high-fat diet, a slight decrease was followed by an increase in the percentage of reserve material present. It would appear that, on the whole, high-protein and high-fat diets have very little, if any, influence upon the polysaccharide storage.

TABLE 3.—*Influence of diet on polysaccharide storage in* N. ovalis

Showing percentage of each population which approached most nearly to each of the 4 chosen standards (see Fig. 1)

		Starvation	High-carbohydrate diet	High-protein diet	High-fat diet
		%	%	%	%
Beginning ..	Standard 1	12	10	16	16
	Standard 2	50	42	36	36
	Standard 3	32	40	34	34
	Standard 4	6	8	14	14
4 weeks	Standard 1	20	0	10	16
	Standard 2	76	30	30	64
	Standard 3	4	44	38	18
	Standard 4	0	26	22	2
8 weeks	Standard 1	16	2	4	12
	Standard 2	72	30	57	40
	Standard 3	8	38	34	34
	Standard 4	4	30	5	14

Fat Reserves

Lipoid reserves have been found in a large number of protozoa (von Brand, 1935). Consequently, a study of the fat reserves of the protozoa parasitic in the cockroach was included in this investigation. Generally, the staining methods employed failed to reveal fat in the form of visible droplets in any of the species being considered. However, after the hosts had been fed on a high-fat or a high-protein diet for six to eight weeks, droplets which stained a bright orange with Sudan III were sometimes present in *Endolimax blattae* and occasionally also in *N. ovalis*. Since the milk-powder used to provide a diet high in protein contained 26.5% of milk fat, it is not surprising that fat storage occasionally occurred in amoebae from hosts provided with this food.

Size of N. ovalis

Since many intestinal protozoa feed directly on the food of the host, it appeared of interest to determine whether or not size changes occur in the parasites when the diet of the host animal is varied. *N. ovalis* is best-suited for studies of this type because it is present more frequently and abundantly than some of the other species and is of larger size.

For each of the four previously mentioned diets, three test groups were used: (1) roaches just collected and not yet placed on a special diet, (2) animals fed on a

given diet (or starved) for four weeks, and (3) hosts placed on a particular diet (or starved) for eight weeks. Fifty specimens of *N. ovalis* from each test group were measured in the manner previously described. There was one exception since in starved animals the number of parasites present was so small that only twenty-five individuals per group were measured. The complete results of these studies are shown in Table 4.

TABLE 4.—*Influence of diet on size (in μ) of* N. ovalis

		Initial	4 weeks	8 weeks
Starvation	Length	85.2 ± 2.3	67.9 ± 2.9	54.5 ± 1.8
	Width	58.2 ± 1.8	48.7 ± 2.5	35.6 ± 1.5
High-carbohydrate diet	Length	73.5 ± 2.0	91.4 ± 3.7	90.0 ± 3.1
	Width	52.2 ± 1.8	67.5 ± 2.9	64.1 ± 2.3
High-fat diet	Length	79.3 ± 1.6	75.8 ± 1.4	67.1 ± 1.7
	Width	56.0 ± 1.5	54.3 ± 1.3	46.6 ± 1.2
High-protein diet	Length	79.3 ± 1.6	87.5 ± 2.5	80.9 ± 2.3
	Width	56.0 ± 1.5	62.7 ± 2.0	55.7 ± 1.7

Ciliates from starved roaches had decreased considerably in size at the end of four weeks and were still smaller after eight weeks. *Nyctotherus* from hosts fed on a high-carbohydrate diet were significantly larger after four weeks but no further increase had occurred after eight weeks. The high-protein diet also produced a certain increase in size to a maximum at the end of four weeks but not quite as much as with the high-carbohydrate diet. A high-fat diet produced very little change in the size of the ciliates for a relatively long period of time, but after eight weeks had elapsed, they were notably smaller than at the beginning.

Number of N. ovalis *per Host*

The experiments described above revealed that the percentage of roaches harboring *N. ovalis* remained essentially the same, regardless of the kind of food provided. However, it was thought worthwhile to determine whether or not the numbers of parasites per host could be influenced by the various diets.

After counts had been made for each series, the results were studied statistically to determine whether or not the increase or decrease in the numbers of *N. ovalis* was significant. The results of these studies are shown in Table 5.

TABLE 5.—*Influence of diet on numbers of* N. ovalis *per host*

	Initial	2 weeks	4 weeks
Starvation	203 ± 43.8	174 ± 28.6	107 ± 11.7
High-carbohydrate diet	203 ± 43.8	347 ± 68.5	539.6 ± 86.5
High-fat diet	203 ± 43.8	145.2 ± 23	139.7 ± 22.6
High-protein diet	203 ± 43.8	166.8 ± 30.5	159 ± 26.3

Several points of interest will be noted: (1) The high-carbohydrate diet was the only one which produced a significant increase in the numbers of ciliates present. The increase was most rapid during the first two weeks, after which time it was very slight. (2) The starved animals alone showed a significant decrease in numbers of *Nyctotherus*. During the first two weeks of starvation, the decrease was small but became statistically significant after four weeks had elapsed. (3) On each of of the other diets, the numbers of *N. ovalis* became somewhat smaller, but in neither case was the decrease significant.

DISCUSSION

The experiments reported in this paper were undertaken in order to determine the influence of starvation and of several different diets on certain intestinal protozoa of the cockroach. The starvation experiments resulted in the complete elimination of *Endolimax blattae* and *Lophomonas blattarum* while *Endamoeba blattae* showed a marked decrease in infection rate. There was little or no decrease in the number of host animals infected with *Nyctotherus ovalis* and *Lophomonas striata.* Varied results have been obtained by other investigators in similar experiments. Cleveland (1925b) found that termites starved for six days lost their infection with *Trichonympha* and if starved for eight days lost *Leidyopsis,* another type of intestinal flagellate. Hegner and Eskridge (1937a) concluded that trichomonads are not easily eliminated from rats by depriving the host of food. In the present study starvation of roaches resulted in a decrease in the size and numbers of *N. ovalis,* as well as in the amount of paraglycogen stored by this ciliate. These results were comparable with those obtained by Harding (1937b) when the ciliate, *Glaucoma,* was starved and by Mowry and Becker (1930) with the ciliates of ruminants.

Except for *N. ovalis,* the incidence of infection of the parasites studied remained highest when the roaches received food having a high-carbohydrate content. Sassuchin (1931) reported similar results in his work on *Blatta orientalis.* Hegner and Eskridge (1937b) observed that a diet high in carbohydrates created a favorable environment for the amoebae in the caecum of the rat, and Mowry and Becker (1930) reported a doubling of the numbers of rumen infusoria with the addition of cornstarch to the ration of goats receiving only hay. A high-carbohydrate diet resulted in an increase in the size, amount of stored paraglycogen, and numbers per host of *N. ovalis.*

Saccuchin (1931) reported that the intestinal protozoa of the cockroach, *B. orientalis,* showed a decreased percentage of infection when a high-protein diet was supplied. On the other hand, Morris (1936) stated that meat or other high-protein food, such as milk powder, was of distinct advantage in maintaining good infections with *Endamoeba blattae* in all the common species of cockroaches. Hegner and Eskridge (1937b) observed that a diet high in protein but low in carbohydrates modified the caecal contents of rats so that amoebae were no longer able to live in this location. Hegner (1923) and Ratcliffe (1930) reported a reduction of caecal flagellates in rats on a high-protein diet, while Kessel (1929) and Kessel and Huang (1926) reported a decrease in the numbers of amoebae when an exclusive milk diet was given to rats, monkeys, and children. In the present work, when a high-protein diet was supplied to the roaches, the flagellates studied showed a decreased incidence as did those in the experiments described by other investigators. The amoebae of the cockroach were not eliminated by this type of diet, as were those of the rat. The protein diet produced no significant change in the numbers of *N. ovalis* per host and had little influence on the parasite's storage of polysaccharides. There was, however, an increase in the size of *N. ovalis* on this kind of diet.

Few papers have reported studies of the influence of high-fat diets, on the persistence of intestinal protozoa. In the present investigation this type of diet resulted in a decreased infection rate for flagellates and *Endamoeba blattae* but caused little change in the percentage of infection with *N. ovalis.* Providing the host with a high-fat diet did not significantly change the number of *N. ovalis* per host nor the

amount of its polysaccharide reserves, but the parasites became somewhat smaller in size. Fat storage was not frequently observed in any of the protozoan species considered but was induced in *Endolimax blattae* and occasionally in *N. ovalis* when a high-fat diet was supplied.

That modifying the diet of a host has a decided influence on its intestinal fauna has been clearly demonstrated by the results discussed in the foregoing pages. Several theories have been advanced as to the factors responsible for the changes observed. A high-carbohydrate diet in most cases is more favorable and a high-protein diet may be unfavorable. Hegner (1937) mentioned several possible factors that might be responsible for these results: (1) changes in hydrogen-ion concentrations, (2) the character of the bacteria resulting from different kinds of diets, and (3) the protozoa may actually require carbohydrate as a food element. Becker and Morehouse (1937) expressed the belief that reduced oöcyst production by the coccidian, *Eimeria miyairii,* when host rats were given skim-milk powder and certain other diets was due to the absence of a special nutrition-factor from the diet, rather than to a positive arrester of the parasite's development. The method of nutrition of the protozoa may have a limiting influence, especially on holozoic species. For example, Lund (1930) found that all of the intestinal protozoa of the termite, *Termopsis,* died very quickly when the host was given a diet of soluble materials. One of the reasons to which he attributed this was the fact that most of the protozoa of *Termopsis* are holozoic and if all of the food is in solution they are not able to ingest enough to keep alive.

Another possible explanation for the results obtained is that, because they live under anaerobic or partially anaerobic conditions, the intestinal parasites have a high-carbohydrate metabolism and consequently thrive on a diet rich in this kind of food. This view is supported by the fact that, in practically every case described, the intestinal forms were present more frequently and in larger numbers when the host was fed on a high-carbohydrate diet.

A condition that is unfavorable for one species of protozoön may not produce the same effect on other species. It is, therefore, likely that the factors responsible for the changes observed are not the same in every case but that under various circumstances many different ones may be concerned.

SUMMARY

1. The American cockroach, *Periplaneta americana,* was maintained under several conditions, including starvation, high-carbohydrate, high-fat, and high-protein diets.

2. The effect of these diets on the persistence and on the polysaccharide and fat reserves of five species of intestinal protozoa of the cockroach was observed.

3. Quantitative studies were also made to determine the influence of the diets on the polysaccharide storage, the size, and the numbers of *Nyctotherus ovalis.*

4. Host starvation lowered the incidence of infection for most of the species studied, while a high-carbohydrate diet maintained it at a relatively high level. However, the persistence of *N. ovalis* was apparently not influenced by the diet of the host.

5. Starvation of the host produced a decrease in the size and number of *N. ovalis,* as well as in the amount of paraglycogen stored by the species.

6. A high-carbohydrate diet resulted in an increase in the size, amount of stored paraglycogen, and numbers of *N. ovalis*.

7. Diets high in fat or protein, in general, exerted very little influence on the intestinal fauna of the cockroach.

8. Several factors which might be responsible for the results obtained are discussed.

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THE EFFECT OF NICOTINE-BENTONITE AND OF CERTAIN PHYSICAL STATES UPON THE EFFICACY OF PHENOTHIAZINE AGAINST NEMATODES IN FOWLS

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Phenothiazine has received greater study than any other anthelmintic used in veterinary medicine, although its usefulness in this field was discovered only a few years ago. This intensive study has led to many practical applications. However, the field of possible usefulness of phenothiazine is far from completely explored. Additional investigations are needed to determine the relative susceptibility to phenothiazine medication of the various *Trichostrongylus spp.* which occur in sheep, to assess more accurately the practical value of using somewhat larger doses of phenothiazine in sheep, to evaluate the efficacy of mixtures of certain drugs with phenothiazine, and to determine if phenothiazine-salt mixtures are useful for the control of parasites in animals other than sheep. For the most part, this article explores the efficacy of a mixture containing phenothiazine and nicotine-bentonite in chickens.

HISTORICAL

The anthelmintic value of an especially prepared nicotine-bentonite compound (containing 5% nicotine expressed as alkaloid) for the removal of *Ascaridia galli* from chickens was first described by Taylor and Galloway (1936). Nicotine products, described earlier, which contained the alkaloid or its salts merely adsorbed on various earthy materials or ground drugs, have not proved quite so satisfactory for use in poultry, and may react quite differently when mixed with phenothiazine. Levine (1936) reported that nicotine-bentonite removed all of the *Ascaridia galli* present in 45 pullets at the time of treatment. Apparently no other tests with this particular nicotine-bentonite preparation have been published. However, data collected at this laboratory by Dr. Ray M. Batchelder show that 94 chickens eliminated 1164 *Ascaridia* (98 per cent of the total present) within 48 hours of treatment with tablets containing 25 grains of nicotine-bentonite. Furthermore, when Dr. Batchelder administered one-half of the above mentioned tablets to each of 46 chickens weighing 2 pounds or less, 828 *Ascaridia* or 91 per cent of the total present were removed by the treatment. Nicotine-bentonite is not effective for the removal of other parasites from the chicken.

Various investigators report that phenothiazine possesses only slight efficacy against *Ascaridia galli,* but that it does possess considerable efficacy for the removal of *Heterakis gallinae.* McCulloch and Nicholson (1940) reported that 0.05 to 0.5 grams of phenothiazine, per bird, would destroy 95 to 100 per cent of the *Heterakis* present. Certain of the *Heterakis* were found by these workers in the ceca at necropsy, but it was claimed that these worms were dead as proved by the absence of movement when they were stimulated with warm water. Apparently, this method of detecting the absence of life as employed by McCulloch and Nicholson was inadequate since later investigators found that all *Heterakis* retained following treatment

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were alive. However, the number of worms eliminated in the investigations of McCulloch and Nicholson, as well as the tests reported by Allen, Olivier and Peterson (1942), Roberts (1940), Knowles and Blount (1941), as well as Guthrie and Harwood (1942), indicate that phenothiazine in adequate doses is effective for removal of *Heterakis gallinae* from chickens.

MATERIALS AND METHODS

The drugs employed were obtained from the usual commercial sources. Since the conditions of dosing varied from experiment to experiment, these will be reported in detail for each test.

The chickens employed were purchased locally and came from three flocks. All birds were cockerels, and all birds from the same flock were of the same age. The birds from one flock were Brown Leghorns, from the second Anconas, and from the third a mixture of White Wyandottes and Silver Lace Wyandottes.

Because most of the birds employed were of a Mediterranean breed, and easily upset by handling, slight loss of weight during the course of an experiment is not significant. The safety of the drug must rest largely on the size of dose necessary to produce death, as shown below. Unfortunately, the Brown Leghorn cockerels contracted a contagious disease of the respiratory tract, which complicates somewhat the interpretation of results. Since birds obviously suffering severely from respiratory disease were not used, this factor was reduced to a minimum, but in one or two instances, noted herein, the effects of the extraneous disease may have been important.

Only *Ascaridia* and *Heterakis* were studied carefully in these experiments, but it was noted that these birds were also infected with *Capillaria spp., Davainea proglottina, Hymenolepis carioca, Raillietina cesticillus,* and *Choanotaenia infundibulum.* Before treatment, birds were placed in individual cages, in which they remained until they were killed. Every 24 hours intervening between treatment and death, the feces eliminated by each bird were collected, washed in screens, and searched for worms. Two to four days after treatment, the birds were killed, and the intestinal tracts searched for worms. The efficacy of the treatment was determined in the usual manner. The objection that dead worms may be destroyed by digestion, though valid with some species of parasites, is easily refuted in case of *Heterakis gallinae* and *Ascaridia galli* by direct observation of the worms. Also, in a portion of these tests untreated controls showed that only an insignificant number of the parasites are eliminated in the absence of treatment.

This method of conducting anthelmintic research, which is known as Hall's critical test, has been criticized of late by a few investigators because certain statistical methods have not been applied to results obtained by it. However, Hall's critical test enables one to determine with precision the size of a population exposed to a given amount of the drug, and the effect of the drug on that population. Therefore, one basis of variation inherent to all other methods of studying anthelmintics is removed.

Nevertheless uncontrolled variables exist in the experiments which are reported in this article, namely, the number of worms in each bird and the relative efficacies of the same treatment in each of several birds. Statistical control of these variables may be desirable, but it is difficult to achieve as acknowledged by the authors of Joint Publication No. 4 (1943: apparently B. G. Peters and R. A. Fisher). On the other

hand, the extensive experience of the Zoological Division, United States Bureau of Animal Industry, which has introduced many useful anthelmintics, indicates that the results of the critical test may be assessed with a very practical degree of precision by direct inspection. On this basis, the recent attempts to discredit reports of anthelmintic research which were conducted by methods of established usefulness are deprecated. The writers find that most reports dealing with phenothiazine as an anthelmintic are useful, interpretable and reasonably consistent. The inconsistent results reported by Peters, Leiper and Clapham (1941), but refuted in Joint Publication No. 4 (1943), were obtained by ignoring certain well-known characteristics of the helminths studied, namely, the rates of acquisition and development of these parasites. Consequently, the writers believe that Hall's critical test is a method of established value when suitably employed. Because helminths vary greatly in structure, habits and habitats, other methods, which may involve analysis of variables, are likewise valuable, but need not be discussed at this time.

EXPERIMENTAL RESULTS

As the first three experiments were orientation tests, a summary of the results obtained, rather than the usual presentation of individual records will be reported.

Experiment 1. In this test 0.25 grams of phenothiazine and 0.56 grams of nicotine-bentonite were placed in each of 11 hard gelatin capsules, and given to each of 11 Brown Leghorn cockerels weighing from 785 to 1,000 grams each. The birds were killed 48 hours after treatment. Of the 33 *Ascaridia* present, 29 (88 per cent) were removed by the treatment, and of the 190 *Heterakis* present, 131 (68.9 per cent) were removed. All the *Ascaridia* were removed from 9 chickens, and all the *Heterakis* from 3 chickens. From 4 chickens the treatment removed less than 70 per cent of the *Heterakis,* a figure which is often used arbitrarily as the minimum for satisfactory efficacy.

These birds were killed only two days after treatment, but many *Heterakis* were eliminated during the second day after treatment, for instance, 9 out of a total of 12 *Heterakis* present in one bird were eliminated on the second day. This suggests that more of these parasites may have been destroyed and would have been eliminated had the birds been retained longer. In all subsequent experiments the birds were held at least three days before necropsy.

Experiment 2. For this test tablets weighing 1.242 ± 0.034 grams were prepared from a formula containing phenothiazine, 1 part, and nicotine-bentonite, 3 parts. On August 20, 1 tablet was given to each of 7 Brown Leghorn cockerels weighing more than 950 grams each and ½ of a tablet to 5 additional cockerels weighing less than 950 grams. These birds eliminated 90.7 per cent of 150 *Ascaridia* and 73.5 per cent of 1332 *Heterakis* present at the time of treatment. All the *Ascaridia* were removed from only 4 of the 9 chickens harboring these parasites, and all the *Heterakis* from only 2 of 12 chickens. Four of the birds eliminated less than 70 per cent of the *Heterakis* present.

Experiment 3. For this test tablets weighing 1.33 ± 0.029 grams each were prepared from a formula containing phenothiazine, 1 part, and nicotine-bentonite, 2 parts. On August 8, 1 tablet was given to each of 7 birds weighing more than 950 grams and ½ of a tablet to each of 5 birds weighing less than 950 grams. These birds eliminated 94.6 per cent of 111 *Ascaridia* and 93.5 per cent of 650 *Heterakis* present at the time of treatment. All *Ascaridia* present were removed from 7 birds

and all *Heterakis* from 4 birds. One chicken eliminated less than 70 per cent of the *Heterakis* present. Since the formula containing the greater quantity of phenothiazine appeared to be the more effective it was employed in several additional experiments.

Experiment 4. The phenothiazine nicotine-bentonite tablets used in experiment three were scored in the center in order that they might be broken in two approximately equal parts for treating small birds. Each ½ tablet was then weighed and given to a cockerel weighing less than 950 grams. These weights are recorded with other pertinent data in Table 1. The treatment destroyed 84.0 per cent of 94 *Ascaridia* and 73.2 per cent of 1093 *Heterakis.*

TABLE 1.—*Efficacy of one-half of a tablet* containing 1 part of phenothiazine and two parts of nicotine-bentonite for the removal of* Heterakis *and* Ascaridia *from Brown Leghorn cockerels*

Band No.	Weight at time of treatment†	Dose	Number of worms removed		Number of worms found at necropsy		Per cent efficacy	
			Ascaridia	*Heterakis*	*Ascaridia*	*Heterakis*	*Ascaridia*	*Heterakis*
	Grams	*Grams*						
2675	835	0.700	2	5	5	14	28.6	26.3
2695	830	0.670	5	23	0	5	100.0	82.1
2278	935	0.688	1	76	0	0	100.0	100.0
2699	880	0.710	12	49	0	19	100.0	72.1
2697	870	0.704	1	22	1	170	50.0	11.5
2696	915	0.691	1	45	1	43	50.0	51.1
2244	855	0.688	25	48	1	0	96.2	100.0
2682	895	0.660	9	82	1	3	90.0	96.5
2276	870	0.700	16	23	6	4	72.7	85.2
2698	940	0.673	2	28	0	28	100.0	50.0
2648	915	0.680	3	180	0	0	100.0	100.0
2649	740	0.692	2	146	0	80‡	100.0	96.9
Total	...	....	79	727	15	366‡	....	....

* For this experiment six tablets were selected at random and broken in two pieces in the fingers. The halves were then weighed carefully and one-half tablet administered to each of the 12 birds shown in the table.

† These birds were treated August 31, 1942, and were necropsied 3 days later.

‡ Bird No. 2649 developed very severe symptoms of respiratory embarrassment the day after treatment and ate very little throughout the course of this experiment. At necropsy 73 of the 80 *Heterakis* found were discolored and partially digested. The remaining 7, though normal in appearance did not move. The partially digested worms were believed to have been destroyed by the drug and are considered as "worms removed" in calculating the per cent of efficacy. All worms normal in appearance were considered to be alive.

Experiment 5. This experiment was designed to study both the efficacy of the tablet described in Experiment 3, and the comparative efficacy of the individual ingredients used in the manufacture of the tablet. Four birds were selected at random. To the first bird, one tablet was given; to the second, 0.44 gram of phenothiazine (a quantity equivalent to the amount used in the manufacture of the tablet); to the third, 0.88 gram of nicotine-bentonite; and to the fourth, an empty gelatin capsule. Four groups of birds were studied at one time, as more could not be handled simultaneously. However, this experiment was composed of 3 parts, conducted in 3 successive weeks, so that a total of 12 birds received each treatment. Detailed results of the test are presented in Table 2.

The birds receiving the tablet eliminated 96.2 per cent of 131 *Ascaridia* and 83.7 per cent of 1012 *Heterakis.* Since the birds receiving no treatment eliminated only 3.8 per cent of 131 *Ascaridia* and 0.9 per cent of 1142 *Heterakis,* the efficacy of the tablet is apparent. Nicotine-bentonite alone removed 86.8 per cent of 159 *Ascaridia* and 10.1 per cent of 1246 *Heterakis.* Apparently nicotine-bentonite at the dose rate employed possesses very slight efficacy against *Heterakis,* and considerable efficacy against *Ascaridia.* Phenothiazine alone removed 43 per cent of 100 *Ascaridia* and

89.9 per cent of 675 *Heterakis*. These results are in keeping with earlier investigations which indicate that phenothiazine is effective for the removal of *Heterakis* but only slightly effective for the removal of *Ascaridia*.

TABLE 2.—*Comparative elimination of* Ascaridia *and* Heterakis *from Brown Leghorn cockerels which received four different treatments*

Band No.	Weight at time of treatment* (grams)	Date of treatment (1942)	Number of worms eliminated		Number of worms found at necropsy		Per cent efficacy	
			Ascaridia	*Heterakis*	*Ascaridia*	*Heterakis*	*Ascaridia*	*Heterakis*
Birds dosed with tablets†								
2279	1200	Sept. 8	1	26	0	7	100.0	78.8
2283	1360	Sept. 8	10	261	2	2	83.3	99.2
2287	1330	Sept. 8	9	0	0	0	100.0	....
2293	1220	Sept. 8	12	10	0	0	100.0	100.0
2296	1065	Sept. 15	1	28	0	15	100.0	65.1
2300	1000	Sept. 15	34	26	0	2	100.0	92.9
2956	1170	Sept. 15	31	252	1	16	96.9	94.0
2960	1210	Sept. 15	23	66	2	68	92.0	49.3
2964	1260	Sept. 21	0	37	0	48	....	43.5
2968	1265	Sept. 21	1	63	0	7	100.0	90.0
2972	1100	Sept. 21	1	13	0	0	100.0	100.0
2977	1185	Sept. 21	3	65	0	0	100.0	100.0
Total	....	....	126	847	5	165	....	....
Birds dosed with phenothiazine in hard gelatin capsules†								
2282	1365	Sept. 8	0	35	0	0	....	100.0
2286	1290	Sept. 8	3	1	13	0	18.8	100.0
2290	1125	Sept. 8	4	38	4	0	50.0	100.0
2292	1220	Sept. 8	0	28	0	0	....	100.0
2297	1180	Sept. 15	18	79	20	0	47.4	100.0
2953	1400	Sept. 15	2	22	0	21	100.0	51.2
2957	1135	Sept. 15	2	10	5	14	28.6	41.7
2961	1115	Sept. 15	1	69	0	0	100.0	100.0
2965	1160	Sept. 21	5	88	6	3	45.5	96.7
2969	1150	Sept. 21	2	27	1	29	66.7	48.2
2973	1065	Sept. 21	3	116	0	0	100.0	100.0
2978	1285	Sept. 21	3	94	8	1	27.3	98.9
Total	....	....	43	607	57	68	....	....
Birds dosed with nicotine-bentonite in hard gelatin capsules†								
2285	1200	Sept. 8	22	52	5	171	81.5	23.3
2289	1230	Sept. 8	0	3	0	31	....	8.8
2281	1165	Sept. 8	6	2	0	114	100.0	1.7
2294	1440	Sept. 8	7	3	15	140	31.8	2.1
2298	1330	Sept. 15	17	25	0	109	100.0	18.7
2954	1130	Sept. 15	0	4	0	49	....	7.5
2958	1070	Sept. 15	5	3	0	75	100.0	3.8
2962	1175	Sept. 15	3	12	0	15	100.0	44.4
2966	1055	Sept. 21	46	0	0	133	100.0	0.0
2970	1110	Sept. 21	13	5	0	159	100.0	3.0
2974	1090	Sept. 21	0	0	0	42	....	0.0
2979	1050	Sept. 21	19	17	1	82	95.0	17.2
Total	....	....	138	126	21	1120	....	....
Birds dosed with an empty hard gelatin capsule								
2288	1090	Sept. 8	2	1	38	150	5.0	0.7
2280	1200	Sept. 8	0	0	14	189	0.0	0.0
2284	1185	Sept. 8	1	0	8	84	11.1	0.0
2295	1275	Sept. 8	0	2	3	72	0.0	2.7
2299	1085	Sept. 15	0	0	9	51	0.0	0.0
2955	1225	Sept. 15	0	0	9	24	0.0	0.0
2959	1210	Sept. 15	2	3	28	234	6.7	1.3
2963	1295	Sept. 15	0	0	0	213	....	0.0
2967	1025	Sept. 21	0	4	7	33	0.0	10.8
2971	1185	Sept. 21	0	0	0	30	....	0.0
2976	990	Sept. 21	0	0	2	37	0.0	0.0
2980	1340	Sept. 21	0	0	8	15	0.0	0.0
Total	....	....	5	10	126	1132	....	....

* All birds were necropsied 3 days after treatment.
† The tablets employed weighed 1.33 grams, and were made of phenothiazine, 33 per cent; nicotine-bentonite (containing 5 per cent nicotine), 66 per cent; and lubricant 1 per cent. The dose of phenothiazine employed was 0.44 gram, and of nicotine-bentonite, 0.88 gram.

Possibly phenothiazine and nicotine-bentonite possess an additive action against *Ascaridia* since the birds treated with the tablet eliminated 96.2 per cent of the

worms present at the time of treatment, while the birds receiving nicotine-bentonite eliminated only 86.9 per cent of the total present. However, the evidence for additive action is far short of proof since 3 of the birds receiving each treatment harbored *Ascaridia* at necropsy. In these tests the tablet removed 6 per cent fewer *Heterakis* than an equivalent amount of phenothiazine. Furthermore, 7 of the birds receiving phenothiazine alone harbored no *Heterakis* at necropsy, while only 3 of the birds receiving the tablet were negative for the cecal worm at necropsy. This suggests the possibility of interference in therapeutic activity between nicotine-bentonite and phenothiazine, a suggestion which is supported by Mohler (1941). Additional evidence on this point will be presented in experiment 8.

Experiment 6. Since the dosage of anthelmintics is ordinarily regulated by weight of the host, a group of large Wyandotte cockerels weighing from 2.5 to 3 kilograms were obtained, and each was given one of the tablets described under experiment 3. Details of the experiment are presented in Table 3. The treatment removed 93.7 per cent of 202 *Heterakis* and the 2 *Ascaridia* which were present.

TABLE 3.—*Efficacy of a tablet* containing phenothiazine and nicotine-bentonite for the removal of* Heterakis *from Wyandotte cockerels†*

Band No.	Weight at time of treatment, November 10	Number of *Heterakis* removed	Number of *Heterakis* found at necropsy	Per cent efficacy for *Heterakis*
	Grams			
2891	2925	8	0	100.0
2892	2835	0	0	....
2882	2475	13	0	100.0
2884	2925	25	0	100.0
2888	2835	8	10	44.4
2889	3015	35	0	100.0
2890	2880	14	2	87.5
2885	3015	47	0	100.0
2886	2565	36	1	97.3
2887	2250	2	1	66.7
Total	....	188	14	....

*The tablet used contained 1 part of phenothiazine and 2 parts of nicotine-bentonite, plus 1 per cent lubricant. It weighed 1.33 ± 0.029 grams.

† The birds used in this test harbored only 2 *Ascaridia*, one each in birds 2892 and 2890. Both worms were removed by the treatment.

Experiment 7. Phenothiazine sufficiently fine to pass through a screen of 12 meshes to the inch, but so coarse that it was retained by a screen of 16 meshes to the inch was obtained. This material was made into tablets similar to those described in experiment 3. One tablet was given to each of 10 Ancona cockerels. The results of this experiment are presented in Table 4. As the tablet removed 95.2 per cent of 62 *Ascaridia* and 92.3 per cent of 889 *Heterakis*, there is little indication that the coarseness of the phenothiazine in any way affected the efficacy of the tablet.

Experiment 8. As both phenothiazine and nicotine-bentonite are frequently administered to chickens in mixtures of feed, an effort was made to study the mixture of the two compounds when administered in this manner. For this purpose 3 grams of phenothiazine and 4.5 grams of nicotine-bentonite were mixed with 115 grams of an all-mash feed and placed before 6 cockerels confined in one cage. For purposes of comparison, a similar mixture containing phenothiazine alone was placed before 6 cockerels of the same age and breed. Because chickens cannot readily obtain all of the feed from a metal trough, twice the above described dose was placed before the birds and the amount consumed was determined by weighing back the residue when about ½ the total had been consumed. Thus the dose actually administered

was greater than originally intended. As these birds were treated in groups, individual records are not available for tabulation. This experiment consists of two tests; in the first test Brown Leghorns were used; in the second, Anconas. The six Brown Leghorn cockerels dosed on October 5 with phenothiazine and nicotine-bentonite actually consumed 128 grams of medicated feed or 5.5 grams more than the intended dose. Fifty-two *Ascaridia* and 226 *Heterakis* were eliminated following treatment. As 4 *Ascaridia* and 15 *Heterakis* were found at necropsy, the percentage of efficacy was 92.9 for *Ascaridia* and 93.8 for *Heterakis*. The 6 cockerels dosed simultaneously with phenothiazine alone consumed 120 grams of medicated mash, or 2 grams more than the intended dose. They eliminated 66.7 per cent of 45 *Ascaridia,* and 94 per cent of 200 *Heterakis* present at the time of treatment.

On November 28, 6 Ancona cockerels were given 9.4 grams of phenothiazine and 14.9 grams of nicotine-bentonite in 238 grams of feed. This dose was somewhat

TABLE 4.—*Efficacy of a tablet* containing coarse phenothiazine and nicotine-bentonite for the removal of* Ascaridia *and* Heterakis *from Ancona cockerels*†

Band No.	Weight at time of treatment	Number of worms removed		Number found at necropsy		Per cent efficacy	
		Ascaridia	*Heterakis*	*Ascaridia*	*Heterakis*	*Ascaridia*	*Heterakis*
	Grams						
2785	1815	1	102	0	0	100.0	100.0
2786	2045	0	20	0	2	100.0	90.9
2787	1810	0	13	0	55	100.0	19.1
2788	1665	14	51	0	8	100.0	86.4
2789	1865	2	20	0	2	100.0	90.9
2790	1725	1	21	0	0	100.0	100.0
2791	1975	0	20	0	0	100.0	100.0
2792	1655	19	206	3	1	86.4	99.5
2793	1545	18	265	0	0	100.0	100.0
2794	1940	4	103	0	0	100.0	100.0
Total		59	821	3	68		

* This tablet weighing 1.34 grams contained 1 part of coarse phenothiazine (16 mesh) and 2 parts of nicotine-bentonite to which 1 per cent of a lubricant was added.
† The cockerels were dosed December 18, 1942, and necropsied 3 days later.

excessive. The birds eliminated following treatment all of 57 *Ascaridia,* but only 67.2 per cent of 765 *Heterakis.* Of the total of 251 *Heterakis* found at necropsy 241 were found in one bird which may not have consumed its full share of the medicated mash. The 6 Ancona cockerels dosed simultaneously with phenothiazine alone received 10.4 grams of phenothiazine from 283 grams of mash. They eliminated 88 per cent of 25 *Ascaridia* and 76.1 per cent of 477 *Heterakis.* As 89 of the 114 *Heterakis* recovered at necropsy were in one bird, it is possible that this bird did not eat a sufficient quantity of the mash to obtain a full dose.

Because of the inadequate control of dosage for each bird in these tests involving mass feeding, it is doubtful whether a valid comparison between the efficacy of phenothiazine and of a mixture of phenothiazine with nicotine-bentonite was obtained. However, it may be noted that in both tests phenothiazine alone was at least as effective as the mixture.

Experiment 9. To test the toxicity of phenothiazine mixed with nicotine-bentonite, each of 23 Brown Leghorn cockerels, which seemed to be healthy at the time, were given on August 31 several tablets similar to those described in experiment 3. The birds were confined to individual cages for one week following treatment, and then released in a pen 15 feet by 20 feet. The test was terminated 3 weeks after treatment. Five tablets were administered to each of 6 cockerels weigh-

ing from 970 to 1200 grams at the time of treatment. As all 6 birds gained weight normally, a dose of this size seemed to be relatively harmless. Ten tablets were administered to each of 12 cockerels weighing from 1025 to 1400 grams at the time of treatment. Five days later all but one of the twelve birds had gained weight, indicating that little or no injury had resulted from this dose. However, during the second week after treatment, shortly after the release of these birds in a common pen, one cockerel which had received 10 tablets was found dead. The appearance at necropsy suggested that injuries received while fighting were the cause of death. Twenty tablets were given to each of four cockerels, weighing from 1020 to 1570 grams at the time of treatment. Five days after treatment 3 of the 4 birds weighed

TABLE 5.—*Efficacy of 0.5 grams of "wettable" phenothiazine compared with 0.5 grams of regular commercial phenothiazine for the removal of* Ascaridia *and* Heterakis *from Ancona cockerels*

Band No.	Date of treatment	Weight at time of treatment (grams)	Worms removed by treatment		Worms found at necropsy		Per cent removed	
			Ascaridia	*Heterakis*	*Ascaridia*	*Heterakis*	*Ascaridia*	*Heterakis*
Birds dosed with "wettable" phenothiazine								
	(1942)							
2781	Dec. 8	1975	6	39	0	0	100.0	100.0
2782	Dec. 8	1855	1	114	0	0	100.0	100.0
2783	Dec. 8	1640	1	189	0	0	100.0	100.0
2784	Dec. 8	1980	0	108	0	0	....	100.0
	(1943)							
3586	Jan. 4	1745	24	5	0	0	100.0	100.0
3587	Jan. 4	1735	8	191	5	34	61.5	84.9
3588	Jan. 4	1860	1	72	8	0	11.1	100.0
3589	Jan. 4	1905	1	137	3	0	25.0	100.0
3590	Jan. 4	1845	51	453	17	0	75.0	100.0
Total	....	....	93	1308	33	34	....	....
Birds dosed with regular phenothiazine								
	(1942)							
2777	Dec. 8	2240	0	1	0	0	....	100.0
2778	Dec. 8	1730	0	196	8	1	0.0	99.5
2779	Dec. 8	1950	0	15	0	0	....	100.0
2780	Dec. 8	1800	0	21	2	0	0.0	100.0
	(1943)							
3581	Jan. 4	1665	2	26	0	0	100.0	100.0
3582	Jan. 4	1785	2	58	4	3	33.3	95.1
3583	Jan. 4	1870	2	113	0	0	100.0	100.0
3584	Jan. 4	2000	0	6	0	42	....	12.5
3585	Jan. 4	1675	22	30	0	0	100.0	100.0
Total	....	....	28	466	14	46	....	....

less than on the day of treatment. However, recovery was prompt since all birds gained in weight between the 5th and 7th days after treatment. One cockerel weighing 1190 grams was given 40 tablets, approximately all the crop would hold. As this dose contains 1.76 grams of nicotine alkaloid, it seemed likely that it would prove fatal. However, this bird which weighed 1190 grams August 31, weighed 1010 grams September 5, 1085 grams September 7, 1235 grams September 14, and 1315 grams September 21. Apparently, chickens are able to withstand excessively large doses of nicotine in the form used for manufacture of these tablets.

Experiment 10. A number of substances are used to make suspensions of phenothiazine that may be administered as a drench to sheep and other animals. Some of these wetting and dispersing agents may possibly affect the therapeutic activity of the phenothiazine. Therefore, an experiment was designed to compare the effectiveness of a "wettable" phenothiazine (containing an admixture of 1.3 per cent of algin and gum karaya) with the regular, commercial product. In this

test one-half of a group of Ancona cockerels were treated with 0.5 grams of "wettable" phenothiazine and the remaining half with 0.5 grams of regular phenothiazine. Results are detailed in Table 5. The "wettable" phenothiazine removed 73.8 per cent of 126 *Ascaridia* and 97.5 per cent of 1342 *Heterakis* present at the time of treatment. The regular phenothiazine removed 66.7 per cent of 42 *Ascaridia* and 91 per cent of 512 *Heterakis*. Apparently the wettable phenothiazine was at least as effective as the regular phenothiazine since it removed a greater percentage both of *Ascaridia* and of *Heterakis*.

DISCUSSION

In these tests, mixtures of phenothiazine with nicotine-bentonite proved effective for the removal of *Ascaridia* and *Heterakis* from chickens, and the mixture seems to be reasonably safe for use since a dose of crop capacity did not cause the death of a cockerel. However, this conclusion cannot be extended to other preparations containing nicotine because many nicotine-preparations are far more toxic than the special nicotine-bentonite used in these tests.

The possible effect of the state of particulation of phenothiazine upon its anthelmintic activity has been discussed occasionally because the drug is so insoluble that large particles which present relatively less surface area might prove ineffective. Guthrie and Harwood (1942) found that micronized phenothiazine was no more effective than phenothiazine of regular grind for the removal of *Ascaridia* and *Heterakis* from chickens. A test reported in this paper (experiment 7) suggests that coarsely ground phenothiazine is as effective against *Heterakis* as that which is ground in the usual manner. These results suggest that within reasonable limits the state of particulation of phenothiazine is not important in the anthelmintic action of the drug when used in chickens.

In the course of these experiments, and of similar experiments reported by Guthrie and Harwood (1942), as well as Harwood and Guthrie (1944), several doses of phenothiazine were used; doses of 0.15 gram of phenothiazine per bird removed 57.5 per cent of 1666 *Heterakis* present in 20 Brown Leghorn cockerels (Guthrie and Harwood, 1942); doses of 0.2 gram per bird removed 60.8 per cent of 926 *Heterakis* present in 6 Ancona cockerels (Harwood and Guthrie, 1944); doses of 0.44 gram per bird removed 89.9 per cent of 675 *Heterakis* present in 12 Brown Leghorn cockerels; and doses of 0.5 gram per bird removed 95.6 per cent of 1853 *Heterakis* from 18 Ancona cockerels. These results indicate that the efficacy of phenothiazine for the removal of *Heterakis* is correlated with the dose, and that a dose of 0.5 gram per bird approaches the optimum. Doses higher than 0.5 gram per bird should be used cautiously since Nicholson and McCulloch (1942) found that repeated doses of 1 gram of phenothiazine per bird affects laying hens deleteriously.

Although nicotine-bentonite possesses slight efficacy against *Heterakis,* phenothiazine alone was more effective for the removal of this parasite than mixtures of phenothiazine with nicotine-bentonite in the three tests described under experiment 5, as well as in the two tests described under experiment 8. In some instances the difference was very slight. Six and two-tenths, 4.9, 14.4, 0.2 and 8.9 per cent more *Heterakis* were removed from birds treated with phenothiazine than from similar birds treated simultaneously with mixtures of phenothiazine and nicotine-bentonite.

The probability that the mean difference (6.92 per cent) was due to chance is less than 5 per cent. Therefore, it is likely that bentonite, which is used extensively for making suspensions of phenothiazine, depresses slightly the anthelmintic activity of phenothiazine. However, in the case of *Heterakis* the slightly lower efficacy of the mixture is not important.

Considering the differences in technique employed, our results are in very good agreement with the results obtained by other American workers (Allen, Olivier and Peterson, 1942; McCulloch and Nicholson, 1940), but foreign workers (Roberts, 1940; Knowles and Blount, 1941) have generally obtained poorer results. Possibly these differences in results may be due to differences in the techniques employed. However, differences in the character of the phenothiazine employed may possibly explain the different degrees of efficacy obtained since Roberts, as well as Knowles and Blount, used phenothiazine containing 5 per cent of a phenol. Apparently, the effect of admixtures of phenols upon the anthelmintic efficacy of phenothiazine has not been studied.

SUMMARY

1. To investigate the possible usefulness of a mixture containing phenothiazine and nicotine-bentonite over 100 naturally infected cockerels of three breeds were employed. A tablet weighing 1.33 ± 0.29 grams proved effective for the removal of both *Ascaridia* and *Heterakis* from chickens. Furthermore, a dose of 40 tablets did not prove fatal to 1 cockerel weighing 1190 grams, although, this dose contained 1.75 grams of nicotine.

2. The use of coarse ground phenothiazine, or of micronized phenothiazine did not affect appreciably the value of the compound for the removal of *Heterakis* from chickens.

3. A "wettable" grade of phenothiazine proved as effective as ordinary commercial phenothiazine for the removal of *Ascaridia* and *Heterakis* from the chickens used in these tests.

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THREE MONOGENETIC TREMATODES FROM MARINE FISHES*

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This paper is a description of two new monogenetic trematodes found on the gills of marine fishes in the region of Tortugas, Florida, and a redescription of another found both at Tortugas and Bermuda. The trematodes from Tortugas were collected in 1930–1932 by Dr. H. W. Manter while at the Tortugas Biological Laboratory of the Carnegie Institution of Washington, D. C. The Bermuda specimens were collected by Dr. F. D. Barker some years ago and were made available through the kindness of the General Biological Supply House.

Specimens were collected by scraping the gills of fishes in dishes containing sea water. They were killed in formal-alcohol-acetic solution under slight pressure of a cover glass, preserved in 70% alcohol, stained with Delafield's hematoxylin, and mounted in balsam.

The writer wishes to express thanks to Dr. H. W. Manter, University of Nebraska, under whose direction these studies were made, and to Dr. Emmett W. Price for certain suggestions.

Family HEXOSTOMATIDAE Price, 1936

Hexostoma macracanthum n. sp.

(Figs. 1–5)

Description: Body 3.57 to 4.25 mm long by 0.77 mm in greatest width, anterior end tapering and pointed but broadening out greatly near level of anterior end of ovary (at anterior fourth of body), followed immediately by a slight constriction and again broadening out slightly toward the posterior region. Posterior portion of body with four pairs of suckers, the anteriormost pair measuring 0.220 mm long by 0.318 mm wide, while the smaller, posteriormost pair measures 0.140 mm long by 0.220 mm wide. Each sucker with three longitudinal chitinous pieces, a large X-shaped piece in the middle and two broad, flat pieces imbedded in the lateral walls. Posterior end of body with two pairs of hooks, a large pair measuring 0.108 to 0.120 mm long by 0.010 mm in greatest width, and a small pair measuring 0.024 mm long. Mouth subterminal. Oral suckers elliptical, 0.034 to 0.047 mm long by 0.027 to 0.029 mm wide. Pharynx ovoid, 0.060 to 0.068 mm long by 0.030 to 0.036 mm wide. Esophagus with several lateral branches anterior to main bifurcation. Ceca much branched, uniting posteriorly by means of numerous, fine, lateral branches. Genital pore ventral, median. Genital atrium conical, 0.059 to 0.068 mm long by 0.051 mm in greatest width. Testes irregular in shape, post-ovarian, arranged more or less in two rows, 34 to 36 in number. Ovary median, near anterior third of body, a somewhat inverted U-shaped organ. Seminal receptacle near posterior right end of ovary, large, 0.24 to 0.28 mm long by 0.068 to 0.084 mm wide. Vaginal opening dorsal, immediately posterior to genital atrium, provided with a pair of cuticular, denticulated bodies, each measuring 0.060 to 0.066 mm long by 0.017 to 0.021 mm wide. Vitellaria extend from level of cuticular bodies to anterior end of posterior third of body. Eggs 0.103 to 0.187 mm long by 0.044 to 0.051 mm wide; anterior filament 0.028 to 0.040 mm long; posterior filament 0.119 to 0.161 mm long. Excretory pores situated anterior to genital pore on dorso-lateral margins of body.

This description is based on two specimens.

Host: Euthynnus alleteratus (Rafinesque), little tunny.

Location: Gills.

Locality: Tortugas, Florida.

Type: U. S. Nat. Mus. Helm. Coll. No. 36890.

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* Studies from the Zoological Laboratory, University of Nebraska, No. 220.

Comparisons.—Hexostoma macracanthum is most like *H. euthynni* Meserve, 1938 in body length and in the size of the anteriormost pair of posterior suckers. It differs from *H. euthynni* mainly in the smaller size of the posteriormost pair of suckers, the structure of the chitinous pieces of the posterior suckers, the number of testes, and in the lengths of the egg filaments (especially that of the anterior filament). Six other species of *Hexostoma* have been named. In proportion to body length, *H. macracanthum* possesses the largest posterior hooks.

Family Diclidophoridae Fuhrmann, 1928
Subfamily Cyclocotylinae Price, 1943
Cyclocotyla hysteroncha n. sp.
(Figs. 6–8)

Description: Body (including haptor) 1.471 to 2.210 mm long by 0.328 to 0.460 mm wide, anterior end tapering and pointed. Posterior haptor approximately one-fourth total body length, with four pairs of pediceled suckers of approximately equal size, 0.098 to 0.101 mm in diameter. Pedicels of equal length, 0.109 to 0.124 mm. Languette very minute, with one pair of hooks approximately 0.028 mm long (Fig. 7). Skeleton of suckers composed of 9 large chitinous pieces (3 paired and 3 unpaired) and 5 or 6 pairs of small, curved lateral bars (Fig. 8). Musculature of sucker more or less divided into four saucer-shaped regions of which one is well developed while either two or three of the others may be greatly reduced (Fig. 8). As a result the musculature of the sucker is often asymmetrical. Mouth subterminal; buccal cavity with a pair of elliptical suckers, 0.028 to 0.032 mm long by 0.024 to 0.028 mm wide. Pharynx relatively large, 0.068 to 0.076 mm long by 0.044 to 0.052 mm wide. Intestinal ceca uniting at level of anterior edge of posterior haptor, with branches extending into posterior haptor and bases of the pedicels. Genital aperture median, immediately posterior to bifurcation of esophagus. Cirrus armed with 6 to 8 bifid hooks, 0.008 to 0.012 mm long (curvature not considered). Testes 6 to 7, post-ovarian, intercecal, smooth, resembling vitellaria in size and shape. Ovary median, near middle of body. Seminal receptacle large, pre-ovarian. Vitellaria filling most of body from level of genital aperture and extending into the haptor. Vagina lacking. No eggs observed. Excretory system not observed. Scattered pigment spots occur near surface of several specimens.

This description is based on five specimens.

Hosts: Bathystoma striatum (Linn.) (type host), *Brachygenys chrysargyreus* (Günther) and *Haemulon flavolineatum* (Desmarest).

Location: Gills.

Locality: Tortugas, Florida.

Type: U. S. Nat. Mus. Helm. Coll. No. 36891.

Comparisons.—The most distinctive features of *Cyclocotyla hysteroncha* are the small number of testes; the relatively large pharynx; and the presence of hooks on the languette.

C. hysteroncha seems to be most similar to *C. elongata* (Goto, 1894), *C. chrysophryi* (Beneden and Hesse, 1863), and *C. labracis* (Cerfontaine, 1895), but differs from all three in body size, in number of testes and cirrus hooks, and in the presence of one pair of hooks on the languette.

Price (1943) considered *Choricotyle* Beneden and Hesse, 1863 a synonym of *Cyclocotyla* Otto, 1823. The subfamily *Cyclocotylinae* Price, 1943 is distinguished from the *Diclidophorinae* Cerfontaine, 1895 by the presence of sucker-like musculature in the claspers. Llewellyn's recent (1941) review of the family Diclidophoridae places most species in the genus *Choricotyle*. Frayne (1943) redescribed "*Diclidophora cynoscioni* (MacCallum, 1917)" which he placed in the genus *Choricotyle*. Price (1943) placed this species in his new genus *Neoheterobothrium*, characterized by a long, slender isthmus between the haptor and the body, and by the vitellaria not entering into the haptor. The known species both possess a languette with two pairs of hooks. Frayne described the vitellaria of his *C. cynoscioni* as

entering the haptor. A specimen of this species collected from the type host at Beaufort, N. C., by Dr. H. W. Manter agrees with Price's description on this point. It is believed that the genus *Neoheterobothrium* is justified and the new combination, *Neoheterobothrium reynoldsi* (Frayne, 1943) is proposed.

Family Microcotylidae Taschenberg, 1879
Microcotyloides n. gen.
Microcotyloides incisa (Linton, 1910) n. comb.
(Figs. 9–14)

Synonym: Microcotyle incisa Linton, 1910.

Description: Body slender, 3.774 to 4.794 mm long (including haptor) by 0.510 to 0.594 mm wide, rounded at anterior end but more pointed at posterior end. Posterior haptor one-third total body length, with 68 to 72 pairs of suckers; suckers 0.076 to 0.086 mm long by 0.044 to 0.053 mm wide. Each haptoral sucker with seven chitinous pieces (Fig. 13): a median U-shaped piece (a) curved dorsoventrally; two pairs of slender curved pieces (b, b′ and c, c′); and an anterior pair (d, d′). Mouth subterminal. Oral suckers elliptical, 0.076 to 0.085 mm long by 0.047 to 0.051 mm wide; each provided with a septum; inner edge of oral sucker bordered by an extension of a thin, cuticular membrane the edge of which is armed with numerous minute spines (Fig. 12). Pharynx spheroidal, 0.034 to 0.037 mm in diameter. Testes ovoid, post-ovarian, 20 to 30 in number. Vas deferens a large sinuous tube extending forward to near the atrium where it bends backward to enter a cylindrical, muscular prostatic bulb, 0.076 to 0.085 mm long by 0.025 mm in greatest width (Fig. 10). The male tube leads posteriorly from the bulb a short distance then bends forward dorsally to become continuous with the penis sheath. Two straight, rod-like diagonal tubes, the prostatic reservoirs, converge to enter the base of the penis sheath (Fig. 10). Penis long, slender, containing three rod-like chitinous pieces. Genital pore median, 0.238 to 0.255 mm from anterior end of body. Genital atrium bulb-shaped, non-muscular, without spines. Ovary median, near posterior end of anterior half of body; oötype median, well anterior to ovary. Vitellaria in lateral fields, extending into anterior portion of haptor where they converge medianly. Vagina present. Vaginal pore in right lateral margin of body, approximately 0.36 mm from anterior end. Vagina divided into two regions, the distal portion lined with smooth cuticula; the proximal portion with rough, crinkled lining. Eggs ellipsoidal, 0.212 to 0.221 mm long by 0.113 to 0.169 mm wide with very long, coiled anterior filament (2.0 mm or more, if straightened) and a shorter, uncoiled posterior filament possessing a terminal knob-like enlargement.

This description is based on six specimens.

Host: Lutianus griseus (Linnaeus), gray snapper.

Location: Gills.

Localities: Tortugas, Florida, and Bermuda Islands.

Slide: U. S. Nat. Mus. Helm. Coll. No. 36892.

Discussion.—Linton's description of *Microcotyle incisa* is very incomplete but it is sufficient to show he was dealing with the above species (from the same host and locality). Linton's figure of the egg is incorrect at least for a typical egg. Eggs become attached to one another by the anterior filament coiling around the short posterior filament of the egg in front (Fig. 14). The knoblike swelling apparently serves to prevent the eggs from separating.

Diagnosis of *Microcotyloides*

Microcotylidae.—Body flat, elongate; posterior haptor as in *Microcotyle*. Atrial opening median, ventral; genital atrium unspined. Male system with prostatic bulb, two prostatic reservoirs, and slender penis possessing three rod-like chitinous pieces; testes post-ovarian. Vaginal pore in right lateral margin of body. Oötype well anterior to ovary.

Type species: Microcotyloides incisa (Linton, 1910).

Comparisons.—Microcotyloides differs from *Microcotyle* chiefly in the lateral position of the vaginal pore, and in the male terminal organs: chitinous penis, prostatic bulb and prostatic reservoirs.

SUMMARY

Two new species of monogenetic trematodes are described: *Hexostoma macracanthum* and *Cyclocotyla hysteroncha,* from the gills of marine fishes from Tortugas, Florida.

Choricotyle reynoldsi is transferred to the genus *Neoheterobothrium.*

A new genus, *Microcotyloides,* is named and described with *M. incisa* (Linton, 1910) as type species.

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Explanation of Plates

All drawings were made with the aid of a camera lucida. Abbreviations are as follows:

cb	cuticular body	prb	prostatic bulb
d	vas deferens	prr	prostatic reservoir
ga	genital atrium	sr	seminal receptacle
gp	genital pore	t	testis
od	oviduct	ut	uterus
ot	oötype	yd	yolk duct
ov	ovary	v	vagina
p	penis		

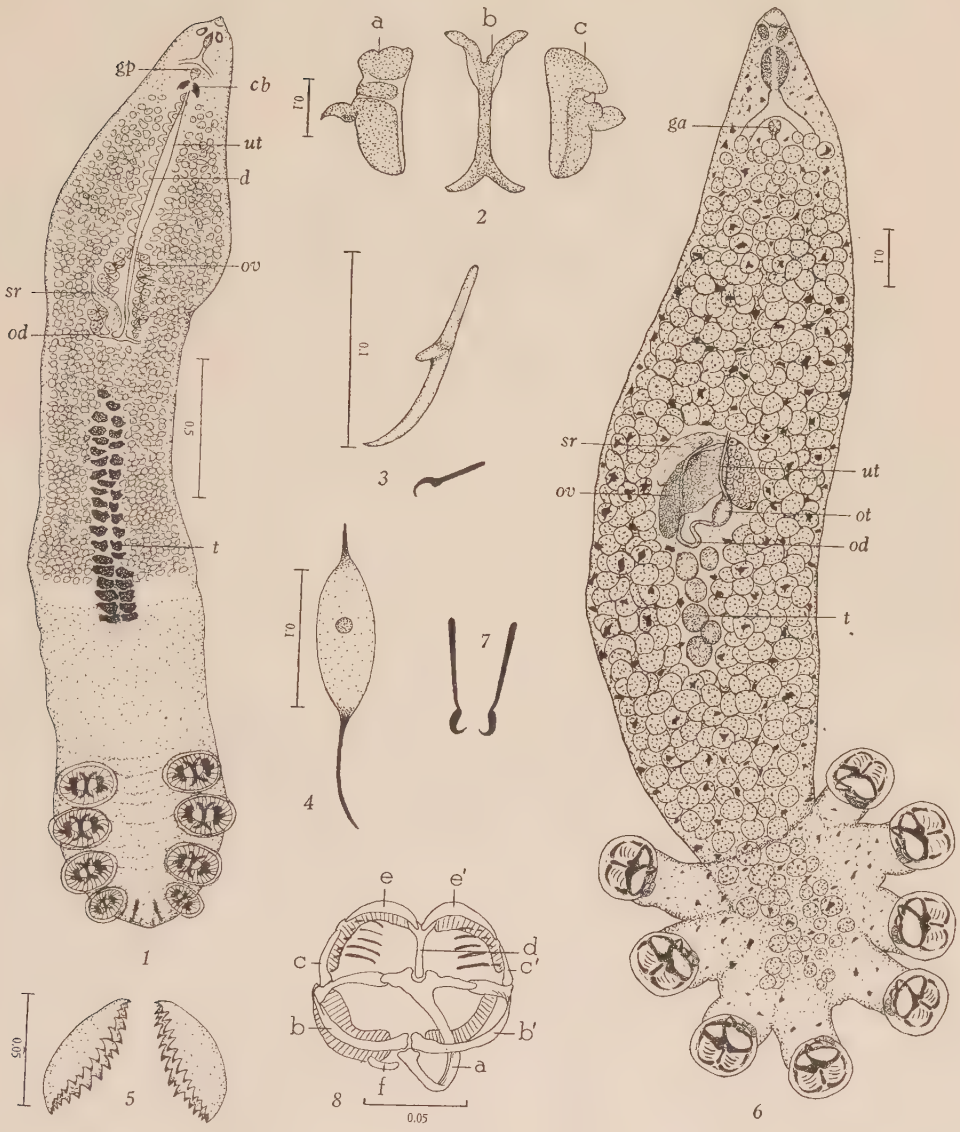

PLATE I

FIG. 1. *Hexostoma macracanthum.* Ventral view.

FIG. 2. Skeletal framework of anteriormost (left) posterior sucker of *H. macracanthum:* (a) inner piece; (b) middle piece; (c) outer piece.

FIG. 3. Posterior hooks of *H. macracanthum,* showing left hook of each pair.

FIG. 4. Egg of *H. macracanthum.*

FIG. 5. Cuticular, denticulated bodies of *H. macracanthum.*

FIG. 6. *Cyclocotyla hysteroncha.* Ventral view.

FIG. 7. Posterior hooks of *C. hysteroncha.*

FIG. 8. Skeletal framework of anteriormost (right) posterior sucker of *C. hysteroncha.* Pieces are labeled as described in text.

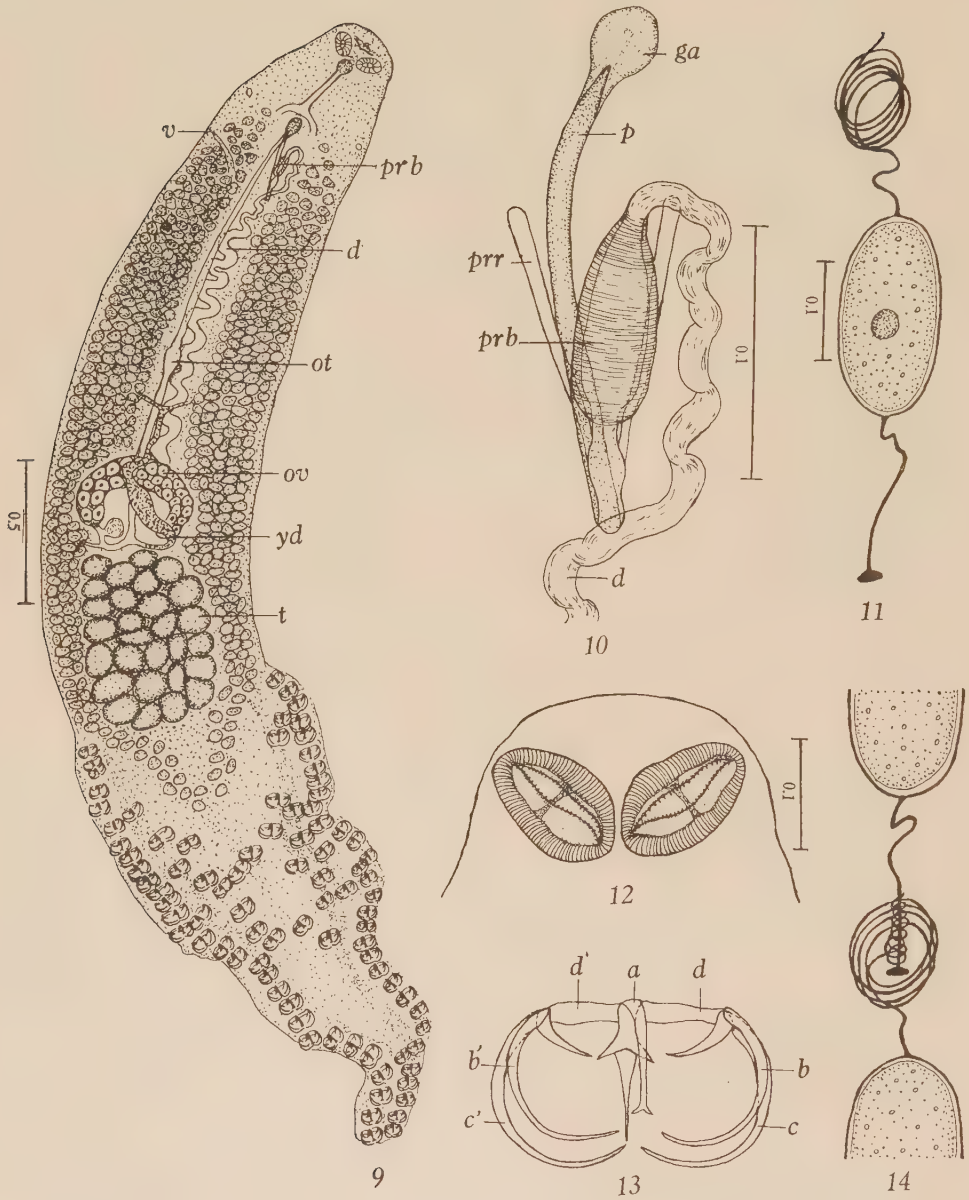

PLATE II

FIG. 9. *Microcotyloides incisa* (Linton, 1910). Ventral view.
FIG. 10. Enlarged portion of male reproductive system of *M. incisa*. Ventral view.
FIG. 11. Egg of *M. incisa*.
FIG. 12. Oral suckers of *M. incisa*. Ventral view.
FIG. 13. Skeletal framework of one of the posterior suckers of *M. incisa*. Pieces are labeled as described in text.
FIG. 14. Anterior filament of one egg of *M. incisa* coiled about posterior filament of another.

SPELOTREMA PSEUDOGONOTYLA N. SP. (TREMATODA: MICROPHALLIDAE) FROM HONGKONG

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In the course of a study of heterophyid trematodes from Hongkong, several specimens belonging to the family Microphallidae were encountered. They are members of the genus *Spelotrema* and differ enough from any known species to be described tentatively as new; the name *Spelotrema pseudogonotyla* is proposed for them.

Spelotrema pseudogonotyla sp. nov.
(Figs. 1–4: all measurements in mm.)

The material studied consists of 50 specimens mounted *in toto* and 8 specimens cut in cross, frontal, oblique and sagittal sections. Since living specimens are not available, and since the large number of eggs covers up most of the internal structures, much of the present study was done on serial sections.

The trematode is ovate, flattened dorso-ventrally in the anterior half, often somewhat concave on ventral surface; body 0.385-0.472 long by 0.184-0.230 wide, with an average of 0.207 by 0.425, greatest width being often at posterior half; cuticle spiny, spines less conspicuous towards posterior end.

Oral sucker terminal, 0.026–0.062 long and 0.026–0.059 wide, average 0.044–0.047; acetabulum distinctly smaller, 0.033-0.043 in diameter, average 0.037, located at almost posterior third of body; genital opening large, somewhat oval in shape, placed to left of acetabulum. Mouth, surrounded by oral sucker, opens into a narrow prepharynx, 0.023 long; pharynx globular, 0.025 long and 0.0245 wide; esophagus slender, 0.106 long and 0.009 wide; intestinal bifurcation at about middle of body; ceca large and short, 0.1 long by 0.024 wide, ending in front of or at level of acetabulum.

Reproductive organs mostly covered by enormous number of eggs; tests two, about 0.05 in diameter, post-acetabular, lying against dorsal wall of body, somewhat obliquely placed opposite each other. From each arises a vas efferens, left one being longer. A short vas deferens leads to an inverted V-shaped seminal vesicle, 0.056-0.082 by 0.036-0.059, average 0.067 by 0.048, placed conspicuously in front of acetabulum, just behind intestinal bifurcation. Prostate glands well developed, surrounding distal portion of seminal vesicle and the short prostatic section; prostate gland mass measures 0.03–0.049 by 0.014–0.039, average 0.039 by 0.028. The prostatic section continues on to form a small but long ejaculatory duct surrounded by a large muscular structure, the male papilla. The latter is bounded on its ventral side by a large and somewhat curved muscular flap, which guards the entrance of the genital pore and is here termed a pseudogonotyl. The male papilla and pseudogonotyl are fused together at their bases. The male papilla narrows and bends dorsally much like a hook, with the male opening at the end. The genital sac, holding the two muscular structures, is spacious. Viewed either dorsally or ventrally, without taking into consideration the distal curved part, the papilla appears as a broad structure, measuring 0.059-0.076 by 0.04-0.053, average 0.069 by 0.047. However, in sections it is about 0.085 in entire length. The pseudogonotyl from the same views measures 0.04-0.056 by 0.013-0.026, average 0.051 by 0.02.

Ovary oval, 0.049-0.066 by 0.04-0.053, average 0.059 by 0.041, somewhat transversely placed just in front of right testis, lying close against the right cecum, near dorsal body wall at about the same level as acetabulum; seminal receptacle not observed. Oviduct turns downward, and at posterior level of testes it turns upward to become the oötype surrounded by Mehlis' glands. The latter mass measures 0.015 by 0.019, located dorsally somewhat on right side and slightly behind testes. Vitellaria in two groups, ventral to male genital glands, between which are the common vitelline duct and the yolk reservoir. Uterus voluminous, containing numerous eggs, occupying entire posterior region up to the tip of the cecum on the right and the posterior limit of genital sac

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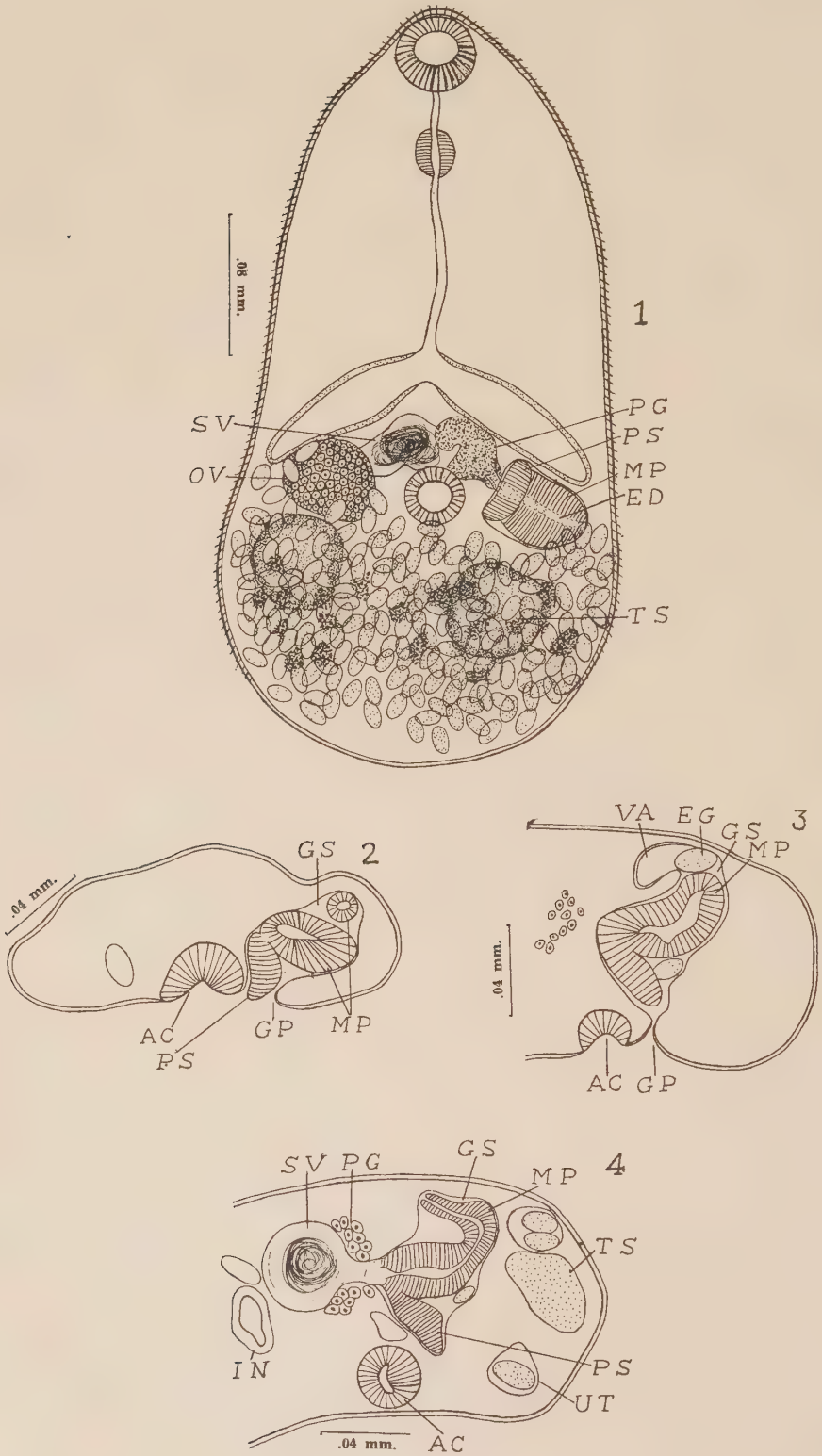
* The University was re-opened in Kukong, Kwangtung, 1942, after the fall of Honkong to the enemy (originally located in Canton but moved to Hongkong when Canton was invaded in 1938). The manuscript was completed in Hongkong but was only recently smuggled to the Free China. The author is now a guest professor in the Chung Cheng Medical College, Yunghsin, Kiangsi Province, China.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IN
PS
UT
.04 mm.
AC

on the left. Proximal end of uterus extends downward from oötype to posterior end of body and then bends upward towards the right, all this portion being filled with spermatozoa. Distal end connected with a short metraterm which opens into the dorsal side of genital sac. Eggs 0.023 by 0.013, oval, embryonated when fully mature, often with a conspicuous rim at operculated end and a minute nodule at opposite end.

Excretory bladder hardly visible in whole mounts. In serial sections it appears V-shaped with a very short stem. Excretory opening terminal.

Location.—Intestine.

Host.—Domestic ducks (*Anas* sp.)

Locality.—Type specimens from Hongkong. The same species was seen by the writer previously in Canton.

Type and cotype specimens.—In the Division of Biology, Lingnan University.

The present species can be distinguished from other species of the genus in possessing a special muscular structure, called here pseudogonotyl, which is attached at the base to the male papilla and guards the entrance of the genital pore. If the distinction among the several genera known at the present time in the family Microphallidae is chiefly in the muscular structure of the male system as elucidated by Rankin (1940), then it may become necessary in the future to reconsider the generic standing of this species. This, however, can be avoided at the present time since, as noted by Tubangui and Africa (1938), our knowledge of the group is still incomplete. Another prominent feature of this species is the typical hook-shaped male papilla.

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Explanation of Figures

Abbreviations

AC—acetabulum
EG—egg
ED—ejaculatory duct
GP—genital pore
GS—genital sac
IN—intestine
MP—male papilla
OV—ovary
PG—prostate glands
PS—pseudogonotyl
SV—seminal vesicle
TS—testis
UT—uterus
VA—vagina

Fig. 1. Adult worm, drawn from mounted specimen with certain structures added from observations on serial sections.

Fig. 2. Cross section of adult worm, showing the position of the pseudogonotyl in relation to the male papilla and the genital pore.

Fig. 3. Cross section of same worm as Fig. 2, showing connection between the genital sac and the vagina, and relation of the genital pore to the acetabulum.

Fig. 4. Oblique section of adult worm, showing relationships of reproductive structures and the hook-shape of the male papilla. A portion of an egg is seen in the genital sac.

SEASON, NUTRITION, IMMUNITY, DRUGS AND X-RAY AS FACTORS INFLUENCING THE COURSE OF A COCCIDIAN INFECTION*

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Adelina deronis is a coccidian parasitic in peritoneal cells of the aquatic oligochaete, *Dero limosa*. During the four-year course of a cytological analysis of the life history of this sporozoan parasite (Hauschka, 1943) extensive ecologic and experimental data were accumulated. Some of these results, bearing mainly on host-parasite relationship, are here combined under one title. The ecologic study is confined to statistics on seasonal incidence. The experimental work is concerned with vitamin effects on host-resistance, controlled infection by cyst-feeding, host specificity and immunity and with attempts to check the parasite through anti-malarial drugs and X-ray.

A self-explanatory diagram of the life cycle reprinted from an earlier publication is given in Text-figure 1.

I. MATERIAL AND METHODS

The seasonal incidence percentages are based on the microscopic examination of 2540 specimens of *Dero limosa* collected over a period of two years from a pond in the University of Pennsylvania Botanical Garden.

Cultures of two species of *Dero* and of five other naid genera (*Nais, Aulophorus, Pristina, Stylaria, Chaetogaster*) were grown in a boiled lettuce medium (Hyman, 1941) with or without the addition of measured quantities of eight B-complex vitamins (Hauschka, 1944). Both infected and parasite-free strains of *Dero limosa* were cultured. Each *Dero* clone was derived from a single worm through budding. Genetic identity of all the animals in a given series was thereby assured.

Comparative growth curves, plotted through weekly counts of individuals and somites in the various cultures, established differences in *Dero* fission rate as conditioned by genetic, nutritional and parasitic factors.

Sporulated *Adelina* oöcysts were fed to parasite-free hosts belonging to six different genera of the NAIDIDAE. The small size and great transparency of the hosts as well as their known culture history practically eliminated errors in the subsequent diagnosis of the infection. The time-relations of the various stages in the coccidian life-cycle and several facts concerning host specificity and natural immunity were thus established.

Attempts were made to rid hosts of their infections by checking the parasites with quinine sulphate, plasmochin naphthoate and atabrin. Aqueous solutions of these drugs, corresponding in concentration to human anti-malarial dosages, were employed in addition to more concentrated and weaker solutions.

Twelve infected hosts were irradiated at weekly intervals with unfiltered X-ray (Pecker-Zephyr apparatus, 100 Kv) ranging in dosage from 35 r to 7000 r.

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* The critical advice of Professor D. H. Wenrich and the expert assistance of Dr. J. T. Farrell, Jr., in the X-ray work is gratefully acknowledged.

Photomicrographs were taken of living parasites through the transparent body walls of chloretonized hosts, without injury to the latter. Exposures ranging from 1/2 to 1/5 sec. were made on 35 mm Kodak Panatomic X film with the aid of a Leitz Micro-Ipso attachment, a Leitz 10× compensating ocular and a Spencer 4 mm achromatic objective. A Sobotka lamp served as light source.

II. RESULTS

1. *Seasonal incidence.*—Forty-eight collections totalling 2540 specimens of *Dero limosa* were taken from the same location in the above mentioned pond during April, May, October and November, 1942, and April and November, 1943. Every worm was examined individually under the microscope.

A total of 1477 animals collected during the spring yielded only 35 infections or 2.3%. During the fall 340 out of a total of 1063, or 31.9% were infected.

Collections taken in December and January were too small to be statistically valuable. During the warm months of the year the oligochaetes build their mucous tubes mainly on the abundant pond weed *Ceratophyllum*. When this plant dies off during December, it sinks to the lower levels of the pond, and some of the worms probably survive the winter in the bottom debris. The more heavily parasitized hosts presumably perish. Vegetative fission ceases almost entirely at winter temperatures and regenerative capacity is no longer capable of counterbalancing parasitic damage to vital tissues. When warm weather returns the surviving worms are largely unparasitized; but many oöcysts may be available for ingestion.

During the spring and summer months *Dero* reproduces sexually in addition to vegetative budding. Since *Adelina deronis* cannot be transmitted through the minute *Dero* egg, which is considerably smaller than a merozoite, an entire new generation of potential hosts arises, greatly swelling the total of uninfected worms.

In summer the infection is widely disseminated. This is accomplished not merely through ingestion of mature oöcysts. The great rise in incidence from 2% in the spring to 32% in the fall is caused largely by transmission from anterior to posterior zoöid through the fission zone during vegetative budding. Evidence for this is the concentration of parasites in the head region of many of the worms examined. On the other hand, infections resulting from cyst-ingestion are most concentrated in the peritoneum opposite the midgut and anterior hindgut.

The efficiency of parasite-transfer through host budding is best shown in laboratory clones. Forty-two days after isolation of eight infected specimens in eight separate dishes the population had risen to 57 animals. Fifty-one of these were infected.

2. *Nutritional factors.*—The B-complex vitamin requirements of two species of *Dero* are described elsewhere (Hauschka, 1944). Eight infected clones of *Dero limosa,* derived from single individuals through budding, began to die off after two months of culture in a B-complex-deficient lettuce medium. In five similar cultures decline was prevented by the addition of a solution of eight crystalline B-vitamins. In spite of rather heavy infection these clones are still growing after seven months of culture. The vitamins greatly increased the lettuce intake of the hosts and thereby aided in maintaining regenerative potency somewhat above the demands of the parasite, so that the worms could undergo fission besides repairing and replacing damaged cells.

While the vitamin-B effect on host growth was very marked, no qualitative difference in the parasitic life cycle was noticed. Since the initial infectious dosage of oöcysts and migration of merozoites across host-fission zones were not controllable, a quantitative comparison of oöcyst yield in tests and controls could not establish the existence of a vitamin effect. Extremely heavy as well as light infections occurred through six generations of a slow-growing clone fed on a vitamin-deficient diet of scalded lettuce alone. Fast-growing clones receiving the B-complex supplement likewise exhibited a marked range in the severity of their infections. From this apparent absence of correlation it seems that the parasite's growth is not necessarily a function of host growth, although conversely host fission frequency is often a function of the parasite population. (See below.)

The quantitative experiments of Becker and Morehouse (1937) and Becker (1940) conclusively demonstrated vitamin effects on *Eimeria nieschulzi* in the rat intestine. A complex of factors, called "coccidibios" by Becker (1940), enhances *Eimeria* oöcyst production, while vitamin B_1 depresses it. "When B_1 is limited, B_6 has been found definitely to promote oöcyst numbers. It may be that 'coccidibios' is a complex of which vitamin B_6 is one of the factors."

In *Dero* the close balance existing between the destructive parasitic factor and vegetative fission rate is evident from a comparison of two culture series: (a) Eight parasite-free isolation lines, each derived from a single animal, showed the following increase after four weeks: 5, 6, 6, 7, 7, 7, 8, 8 (average 6.75; range 5–8). This served as control for (b) eight infected lines, also derived from single worms, which after four weeks contained 1, 2, 2, 4, 5, 6, 7, 8 individuals (average 4.37; range 1–8). One of the infected worms had failed to divide, two had divided only once in four weeks, while the fission rate of the rest was comparable to the control series. Since the animals with fewest offspring were also the most heavily infected, inverse relation is indicated between severity of infection and fission frequency. On the whole, the growth rate of the parasitized series is only 35% below that of the uninfected control.

Starvation of *Dero* does not detectably affect the life cycle and normal cytology or cyst viability of *Adelina deronis,* but results in a loss of balance between damage and repair and consequent dwarfing and death of the host.

The state of nutrition of a host and, to some extent, the physiological age of a given somite, is often expressed by the condition of the chloragogen tissue, which in *Dero* is homologous to the visceral peritoneum. According to Freudweiler (1905), as quoted by Stephenson (1930), the chloragocytes function in the storage of food reserves which are laid down as greenish-yellow globules. Liebmann (1943) states that chloragogue cells frequently contain guanine and draws attention to the work of Hammett (1938) on the growth-activating properties of this purine base in low concentrations.

In worms heavily infected with *Adelina deronis* and consequently undergoing much internal regeneration, there is an abundance of dark brown chloragogen bodies along the intestine (Figs. 1 and 3). Parasite-free hosts have fewer and less refractile chloragogen globules. The animal photographed in Fig. 2 was uninfected until the day before this picture was taken. Here the chloragogen does not stand out as dark spherules and is considerably less concentrated than in Fig. 3. The recent finding of Liebmann (1943), that regeneration in *Eisenia* is entirely under the control of the chloragogen system, appears substantiated by the regeneration-conditioned variations of this system in *Dero limosa.*

3. *Experimental infection of* Dero limosa.—The average duration of the various stages in the life cycle appears in Text-figure 1. The time-relationship was determined through experimental infection of hosts from negative clones. In about 2/3 of the attempts infection was accomplished by a single feeding with crushed worms containing sporulated *Adelina* oöcysts (Figs. 3 and 7).

Within three to four days after cyst feeding large schizonts are recognizable in the peritoneal cells surrounding the posterior midgut (Fig. 8). Due to their lack of refractile inclusions these trophic forms do not yield satisfactory photomicrographs. The stage marked sch 1, in Fig. 5 is a nearly mature schizont showing over twenty merozoites grouped about a central residual mass. It corresponds to stage 11 in Text-figure 1. Three days after maturing of the first generation schizonts, merozoite bundles of the second generation (sch 2, Fig. 4 and stage 14, Text-figure 1) make their appearance. These smaller schizonts give rise to male and female gametocytes, respectively, mating of which may be observed in vivo. In Figs. 4 and 9 recently associated gametocyte pairs predominate (see also stage 17, Text-figure 1). Several somewhat older pairs and trios (2 ♂ attached to 1 ♀) are pictured in Fig. 10. The female in Fig. 5 distinctly reveals its nucleus and nucleolus against the more refractile background of paraglycogen granules in the cytosome.

Fig. 6 represents the condition immediately succeeding fertilization. The nucleus on the lower left is a synkaryon. The microgametocyte remnant on the upper right has been slightly pushed away from the zygote by the recently formed fertilization membrane which in due course gives rise to the oöcyst wall (Fig. 3). Mature oöcysts are usually present 16–18 days after ingestion of the original infectious dose. Oöcysts can hatch in situ and thus the life cycle is reopened without transfer to a new host. For a discussion of the self-limited nature of this cycle the reader is referred to an earlier paper (Hauschka, 1943).

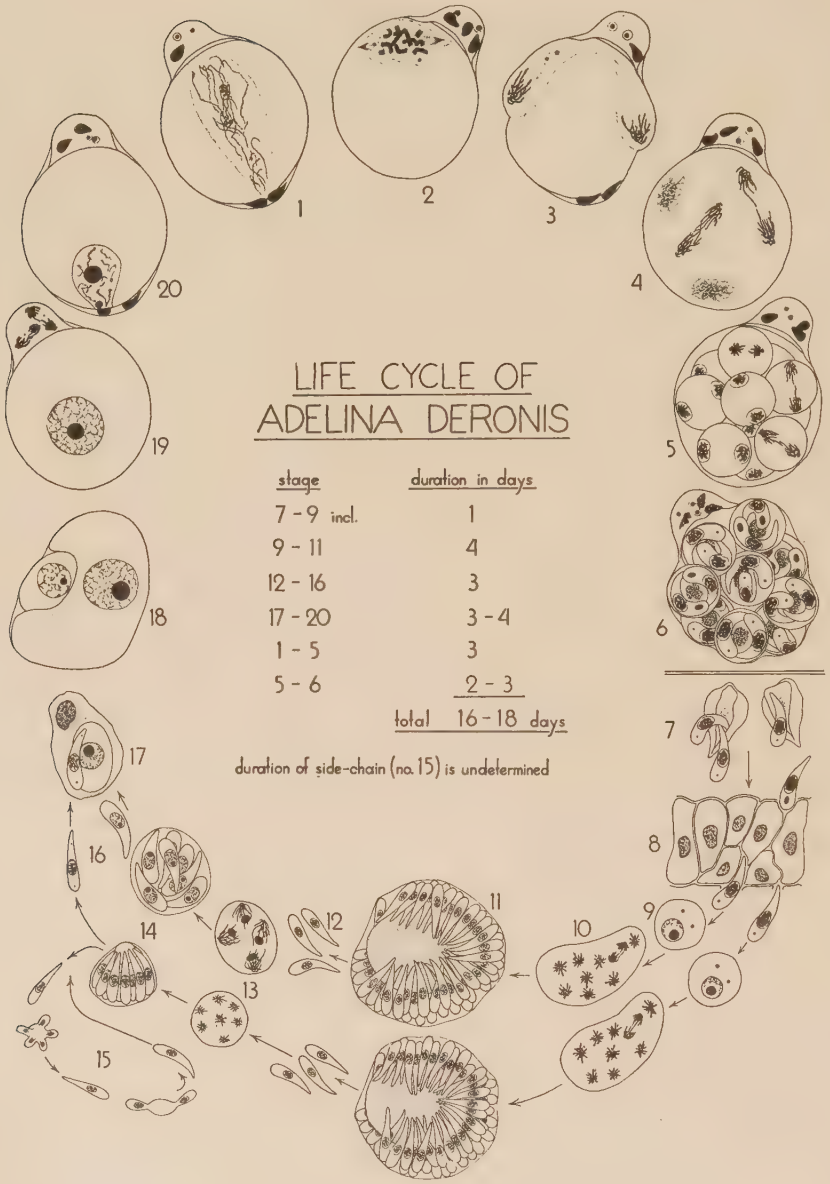
4. *Host specificity and immunity.*—In addition to negative clones of *Dero limosa* several entire cultures and thirty-seven isolated individuals of a larger, unidentified *Dero* species were repeatedly exposed to the infection. The latter species was clearly distinct from *Dero limosa* in size (being about 2.5 times longer and thicker), in length of setae, average number of somites, growth rate and sexual cycle.

This *Dero* must be considered naturally immune to *Adelina deronis* since continued experimental cyst feedings failed to produce positive results and since none of the many freshly collected specimens were infected.

Likewise unsuccessful were attempts to infect five individuals of *Nais* sp., four of *Aulophorus* sp., twenty-two of *Chaetogaster* sp., sixteen of *Pristina longiseta,* a large culture of *Stylaria lacustris* and a culture of *Nais paraguayensis* (available through the kindness of Dr. L. H. Hyman). The oöcysts were readily ingested by many of these oligochaetes, except *Chaetogaster.*

Even within the species *Dero limosa* experimental infection does not always succeed. In spite of heavy dosages of sporulated oöcysts, six out of sixteen infection attempts gave negative results.

To determine whether this relatively high percentage of failure was caused by inadequate procedure, an apparently uninfectable specimen was isolated and the culture was quarantined for two weeks when it contained four genetically identical worms produced by budding. These animals were parasite-free and they and their offspring remained negative for seventy days during which period large numbers of oöcysts were fed to them on five successive occasions.



Explanation of Text-figure 1

1. Zygote, showing homologous chromosomes in process of synapsis.
2. Metaphase of meiotic division of synkaryon.
3. Zygotic meiosis, telophase; this division is immediately followed by a second meiotic division.
4. Early sporogony; karyokinesis not synchronous; this is possibly indicative of the primary sex difference.
5. Late sporogony.
6. Mature oöcyst (double line between 6 and 7 indicates change of host).
7. Spores hatching in host gut; spores are thought to produce male and female sporozoites respectively.
8. Sporozoites penetrating midgut epithelium (stages 7 and 8 may be by-passed in auto-infection).
9, 10, 11. First schizogonic generation, during which no sex difference is apparent.

The actual ingestion of cysts and the hatchings of spores in the intestine were observed microscopically. A photomicrograph (Fig. 2) obtained on the twenty-ninth day of this experiment, shows a sporulated oöcyst (oö) and a recently hatched sporozoite (sp) within the host-gut. The exposure time of 1/5 sec. was not rapid enough to counteract slight blurring due to the twisting movements of the parasite, which may be seen rather faintly near the end of an ingested bristle.

Motile sporozoites were repeatedly observed also in the coelomic fluid of most of the worms in this series, but the half-grown trophic stages of the parasite corresponding to stages 9 and 10 in Text-figure 1 appeared to degenerate within the host cells. None of them ever developed into bundles of merozoites. Instead, they became filled with brownish green globules resembling chloragogen and after a few days were completely replaced by this material.

These happenings in immune host cells, as observed in vivo, correspond with the findings of Tyzzer, Theiler and Jones (1932) in chickens immunized through successive feedings with *Eimeria necatrix* oöcysts. A cytological study of the intestinal mucosa of these birds revealed that "most of the sporozoites are destroyed after invading the gland cells by the failure of the latter to respond in such a way as to favor the growth of the parasite. No evidence has been obtained of any protective mechanism attributable to humoral response."

The observations recorded below in a day by day account were made on lightly chloretonized hosts at a magnification of 400 diameters. After each examination the worms were allowed to recover in spring water and were returned to the culture dish. No animal in this series was lost through injury:

Day 1. A single negative *Dero limosa* isolated in lettuce medium in Syracuse watch glass.
14. Four worms in culture; all negative.
14–16. *First exposure* to cysts from three heavily infected hosts; empty sporocysts observed in gut.
17. *Second exposure* to cysts from seven heavily infected hosts.
18. Oöcysts and sporocysts in gut.
24. All worms negative. One degenerating trophozoite in coelom of one of the animals.
28. All worms negative.
28. *Third exposure* to cysts from ten positive hosts.
29. Oöcysts, sporocysts and free motile sporozoites in host-gut seventeen hours after feeding.
29. *Fourth exposure* to cysts from ten positive hosts.
32. Motile sporozoites in coelom of three hosts; several degenerating trophozoites.
35. Only one host with two motile sporozoites and degenerating trophozoites in coelom. Others negative.
53. Six worms in culture, all negative.

12, 13, 14. Second schizogonic generation, during which sex is differentiated.
15. Waning of microgametocyte-producing schizogony.
16. Young gametocytes; the smaller male shows terminal granules and lacks endosome.
17. Young gametocyte pair in host cell.
18, 19. Growth of gametocyte pairs, and production of microgamete nuclei.
20. Fertilization; one of the four microgametes entering maternal nucleus at fertilization pole.

54–56. *Fifth exposure* to cysts from two heavily infected hosts.

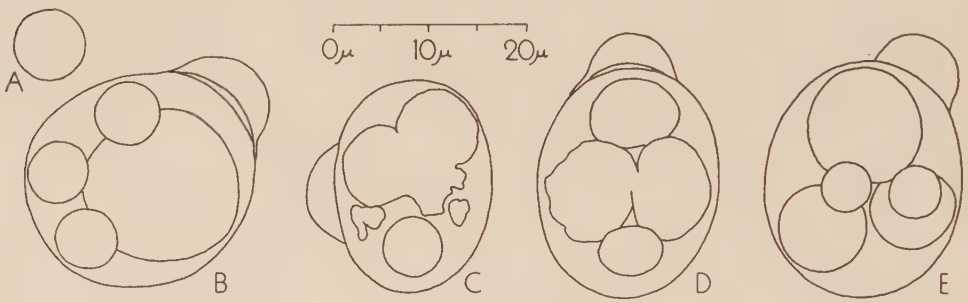
57. One worm with degenerating trophozoites. Five worms with altogether eighteen motile sporozoites in coelom.

70. Nine worms in culture, all negative. No traces even of degenerating parasites. Culture discontinued.

Failure to infect this clone is probably not a matter of dietary deficiency. The culture increased by 900% during the seventy days of observation and was resistant to the infection from the outset. Deliberately starved, parasite-free worms from another strain became infected after only one cyst feeding.

The above clone of *Dero limosa* may therefore be considered immune to *Adelina deronis*. The immunity seems to be natural rather than acquired. In susceptible clones transfer of the parasite from host-generation to host-generation continues unchecked over long periods. The repeated inheritance of the infection does not appear to be accompanied by a gradual decline of the parasitic population.

Adelina deronis is, thus, not merely limited to one host species, but exhibits strain-specificity within this species, as well as marked tissue specificity, since it invades mainly cells of the visceral and parietal peritoneum.



Explanation of Text-figure 2

Oöcyst aberrations induced by X-ray. A. Normal spore. B, C, D, E. Aberrant spores within oöcysts of normal size.

5. *Effects of three anti-malarial drugs.*—Attempts to check *Adelina deronis* through keeping the hosts in solutions of quinine sulphate, atabrin and plasmochin naphthoate were unsuccessful. A large range of concentrations was tried using human anti-malarial dosages as a basis. In most of these solutions the differentially susceptible heads and tails of the oligochaetes degenerated, leaving only the mid-body region with its contained parasites intact. Host fission was greatly reduced or ceased altogether, while the coccidian schizogony and the development of subsequent parasitic stages continued unchecked in twenty-four experiments. Oöcysts were still viable and caused experimental infection after a sixty-hour soaking in a saturated aqueous bath of plasmochin naphthoate which had killed ten infected hosts in from one to three hours. Of the spores, formed while the hosts were immersed in a non-lethal plasmochin medium, 19.1% were of abnormally large size. In controls such aberrations in sporogony amount to less than 10% of total spores formed.

6. *Effects of X-ray.*—Irradiation of four infected worms with 35 r had no apparent effect on sporogony. All the oöcysts in these animals were normal four days after treatment. In three worms rayed with 1000 r, 35.2% of the oöcysts were aberrant one week after exposure. In four hosts rayed with 5000 r the following

incidence of abnormal spore-formation was observed one week later: 67.3%, 77.0%, 89.2%, 100%. The degree and type of aberration is evident from a comparison between Text-figure 1, stage 5 (normal oöcyst) and Text-figure 2.

Seven non-irradiated control-hosts from the same clones, examined at varying intervals after isolation, contained only 3.4% aberrant oöcysts. The above percentages are based on an examination (in vivo) of 474 irradiated and 619 control oöcysts.

Since schizonts continue to appear quite normal and since spore aberrations are first noticed 3–4 days after irradiation, the most susceptible stage is probably the zygote. During early zygotic synapsis the chromosomes are very long drawn out (stage 1, Text-figure 1) and the chances for effective X-ray hits are thereby greatly increased.

A cytological study of this material, now in progress, should reveal whether the unusually large spores are multi-nucleate, polyploid or aneuploid, and whether the few naturally occurring aberrations involve mitotic pictures similar to the experimentally produced ones.

Abnormal oöcysts from irradiated worms may not be viable. Feeding of a large quantity of them to three negative hosts from a susceptible clone did not produce an infection.

Irradiation between 1000 and 5000 r completely inhibits host fission, while control-worms continue to divide. Five of seven hosts irradiated with 7000 r died within two weeks after treatment and probably as a result of it. Preceding death these animals appeared dwarfed.

III. SUMMARY

1. In collections of *Dero limosa* gathered during spring only 2.3% of the hosts were infected with *Adelina deronis*. The incidence rose to 31.9% in the fall.

2. Fission frequency of parasitized clones is only 35% below that of controls.

3. A diet of scalded lettuce does not supply the B-complex vitamins necessary to maintain infected cultures over indefinite periods. The parasites in starved hosts appear normal.

4. A correlation between amount of chloragogen, degree of parasitism and tissue repair is indicated.

5. Experimental feeding of sporulated oöcysts to negative host-clones produced infections in 2/3 of the cases. The time relationship and average duration of the various phases of the coccidian life cycle were established.

6. Host specificity is marked as shown by cross-infection experiments involving oligochaetes of the same genus and five other naid genera.

7. Resistance of one clone of *Dero limosa* to heavy infectious dosages and degeneration of trophozoites in the peritoneal cells of this clone is interpreted as due to natural immunity.

8. Quinine sulphate, plasmochin naphthoate and atabrin do not detectably affect schizonts or gamonts, even at concentrations damaging to host tissues. Plasmochin doubles the frequency of abnormal sporogony.

9. Unfiltered X-ray of 1000–7000 r interferes with host fission and produces aberrant sporogony in from 30–100% of the total zygotes. No effect of X-ray on schizogony or gametogony can be observed in vivo.

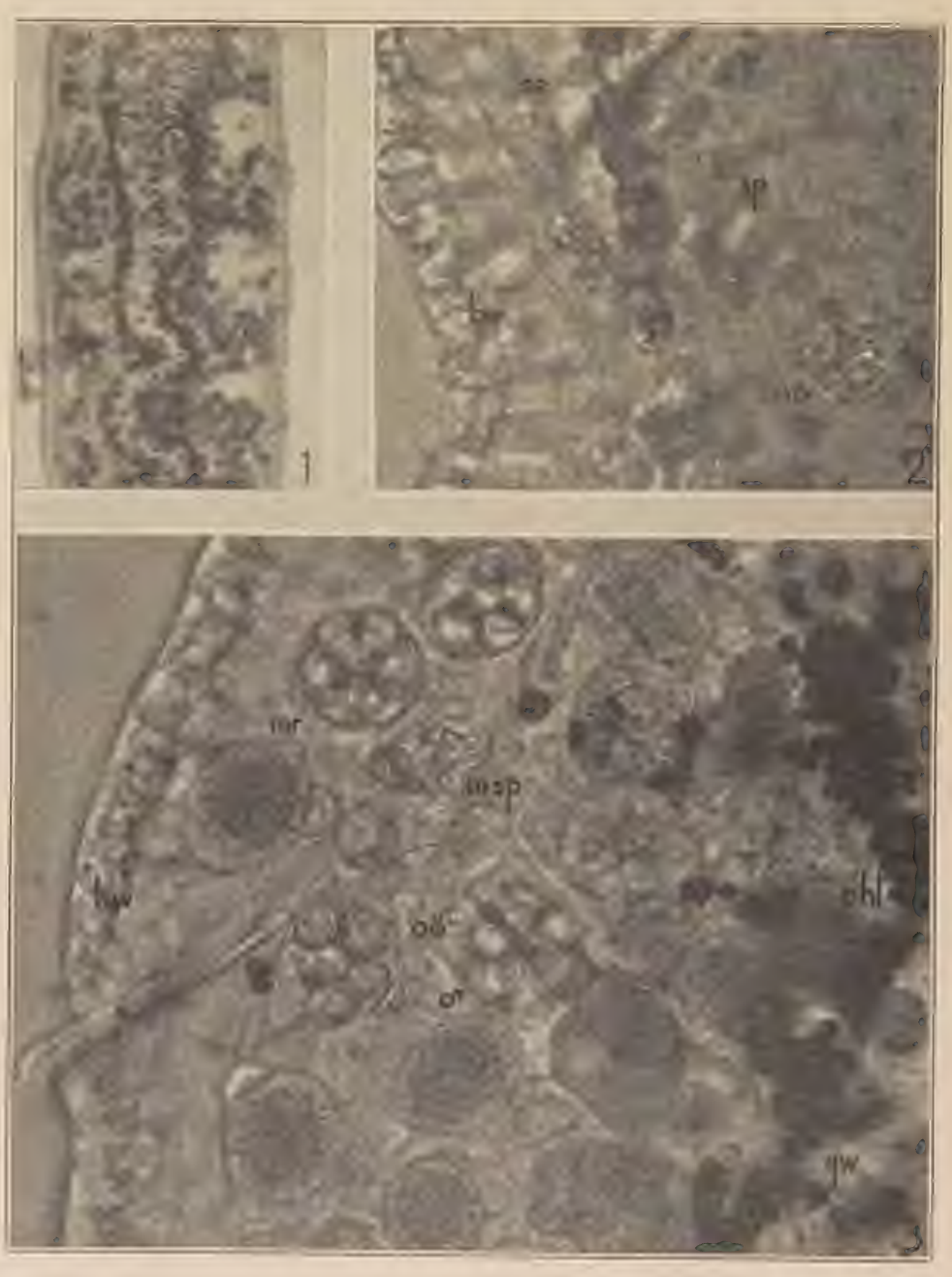


PLATE I

Photomicrographs of the coccidian *Adelina deronis* parasitic in the body cavity of the naid *Dero limosa*. All photographs are of living material, anaesthetized with 0.3% chloretone. Fig. 1 is magnified approximately 130 diameters. In all other figures the magnification is 900 diameters.

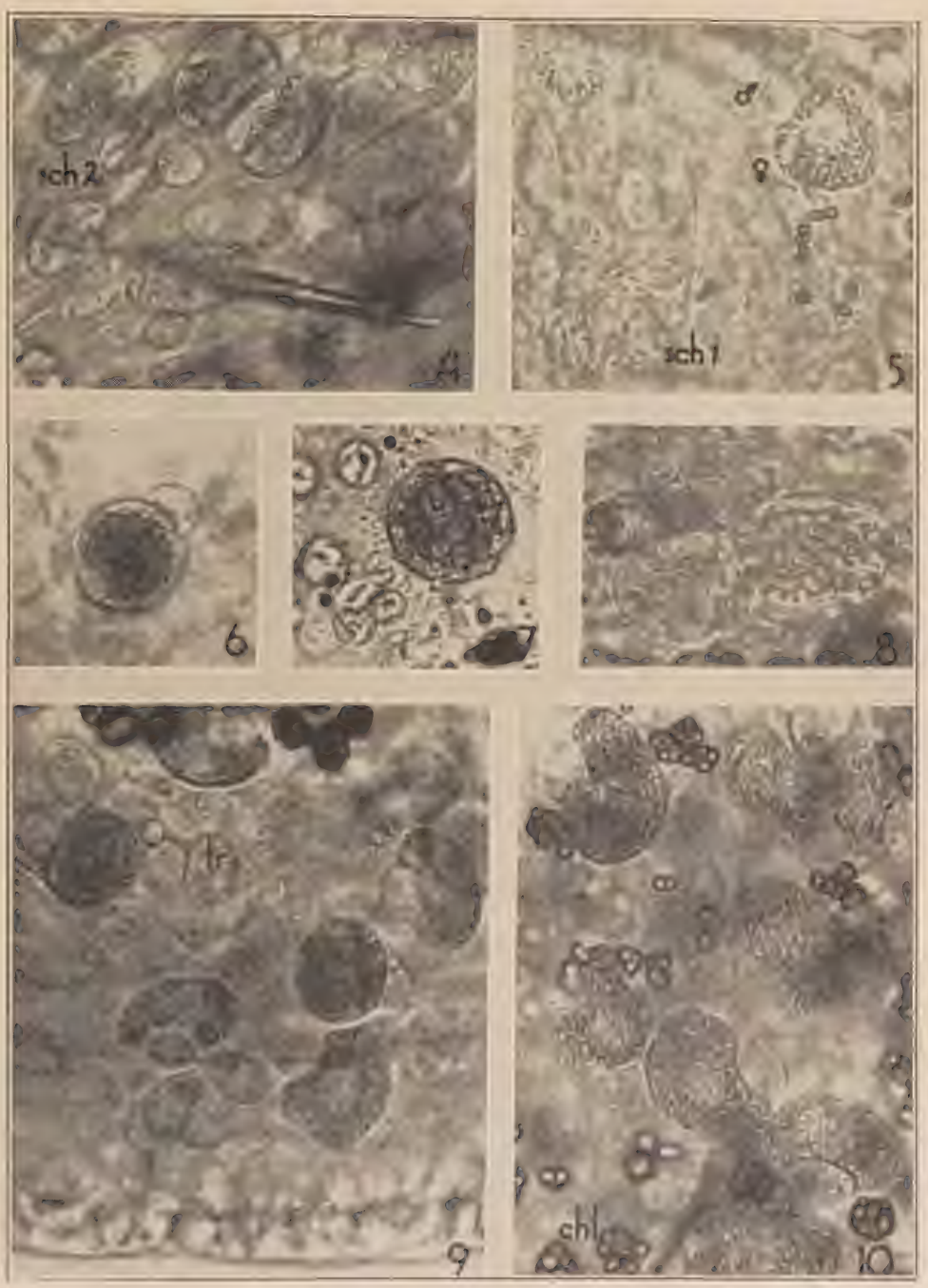


PLATE II

Photomicrographs of the coccidian *Adelina deronis* parasitic in the body cavity of the naid *Dero limosa*. All photographs are of living material, anaesthetized with 0.3% chloretone. The magnification is 900 diameters.

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Explanation of figures, Plate I

Fig. 1. Portion of anterior third of a heavily parasitized worm; dark margin of oesophagus clearly distinguishable due to dense chloragogen material.

Fig. 2. Experimental infection; a newly hatched sporozoite (sp) may be seen free in the intestine; an ingested bristle is pointing to this sporozoite; oö = a recently ingested mature oöcyst, gw = the gut wall, se = a septum, bw = the body wall of the host.

Fig. 3. Appearance of coelom in advanced heavy infection; bw = the body wall, oö = a mature oöcyst, mr = a microgametocyte remnant still attached to mature cyst, msp = mature sporocysts, chl = dark chloragogen granules, gw = gut wall of host.

Explanation of figures, Plate II

Fig. 4. Gametogony and pairing; many young stages in peritoneal tissue; sch 2 = a second generation schizont.

Fig. 5. Half-grown gametocyte pair; sch 1 = a mature first generation schizont.

Fig. 6. Zygote shortly after fertilization.

Fig. 7. Mature sporocysts, each containing two sporozoites and a residual body.

Fig. 8. Half-grown schizonts of the first generation; merozoites are beginning to bulge out.

Fig. 9. Young gametocyte pairs; tr = a trophic pair shortly after association.

Fig. 10. Nearly mature gametocyte pairs and trios; chl = chloragogen granules.

A NEW SPECIES OF *RETORTAMONAS* (PROTOZOA) FROM THE COMMON MOLE CRICKET, *GRYLLOTALPA HEXADACTYLA*

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In 1879 Grassi described a species of *Retortamonas, R. gryllotalpae,* from the European mole cricket, *Gryllotalpa gryllotalpa.* The taxonomy of these flagellates was reviewed and summarized by Wenrich in 1932. No new species has been recorded since then.

The following observations concern a new species which is named *Retortamonas wenrichi,* in honor of Dr. D. H. Wenrich, who has contributed so much to our knowledge of these small protozoa.

MATERIAL AND METHODS

The host from which this species came was an adult male common mole cricket, *Gryllotalpa hexadactyla.* He was caught on August 29, 1942, as he sang lustily in a chamber of his burrow some eighteen inches from the waters of the Patuxent River, at a spot known as Brown's Bridge, Ednor, Maryland. He was transported alive to the University of Pennsylvania, where these observations were made.

At the time, it was not possible to study the living flagellates extensively, so the present data are all recorded from the permanent slides. The material from the rectum was fixed in Schaudinn's fluid, to which was added 5 per cent of glacial acetic acid. The staining was done in Heidenhain's hematoxylin.

OBSERVATIONS

The trophic stages.—The organisms were typical retortamonads. Perhaps the most striking feature of this new form was its extreme length. The longest species described thus far have an over-all range up to 18 μ. The present species has a relatively large body, and a remarkably long, tenuous tail. The body proper ranges from 12.2 μ to 19.0 μ in length, averaging 16.0 μ. Its width range is 3.8 μ to 8.4 μ, averaging 5.9 μ. The tail ranges from a short spike of 3.1 μ to a long filament of 58.5 μ, and averages 23.2 μ. The over-all length, not including the flagella, ranges from 16.7 μ to 76.0 μ, with an average length of 39.2 μ. That this caudal extension was not in the nature of a stiff spike was demonstrated by the fact that it was frequently looped and twisted (Figs. 3, 5).

The anterior end was often seen to terminate in a short, conical projection (Figs. 2, 5). Tailless, precystic forms were occasionally noted (Fig. 6). Many specimens showed a clear area on one side near the anterior end (Figs. 1–5), which was associated with the oral pouch. Some individuals showed the spirally twisted body (Fig. 4), so common in some species of *Chilomastix.*

The mouth area was bordered on one side by the so-called parabasal fiber, and on the other side by the parastyle. These fibers frequently appeared somewhat thicker near their points of origin (Figs. 2, 4). The parabasal fiber, frequently following a

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wavy course (Figs. 3, 4, 5), extended to about the middle of the body proper, then swung across, and often continued some distance back toward the anterior end. This fiber was sometimes accompanied by another stainable thread (Figs. 2, 3, 5), as shown by Geiman (1935) for *Chilomastix* from man, a monkey, and the guinea pig.

The two approximately equal flagella were generally longer than the body, often one and one-half to two times the length of the body proper. Neither showed a marked association with the oral pouch. Arising from separate basal granules, the more anterior one was generally directed forward, while the other frequently took a more backward course, sometimes extending for a short distance along the oral pouch before curving out (Figs. 2, 4, 5). The basal granule giving rise to the second flagellum was also the point of origin for the oral fibers (Figs. 1, 2, 4) but these associations were not always easy to discern.

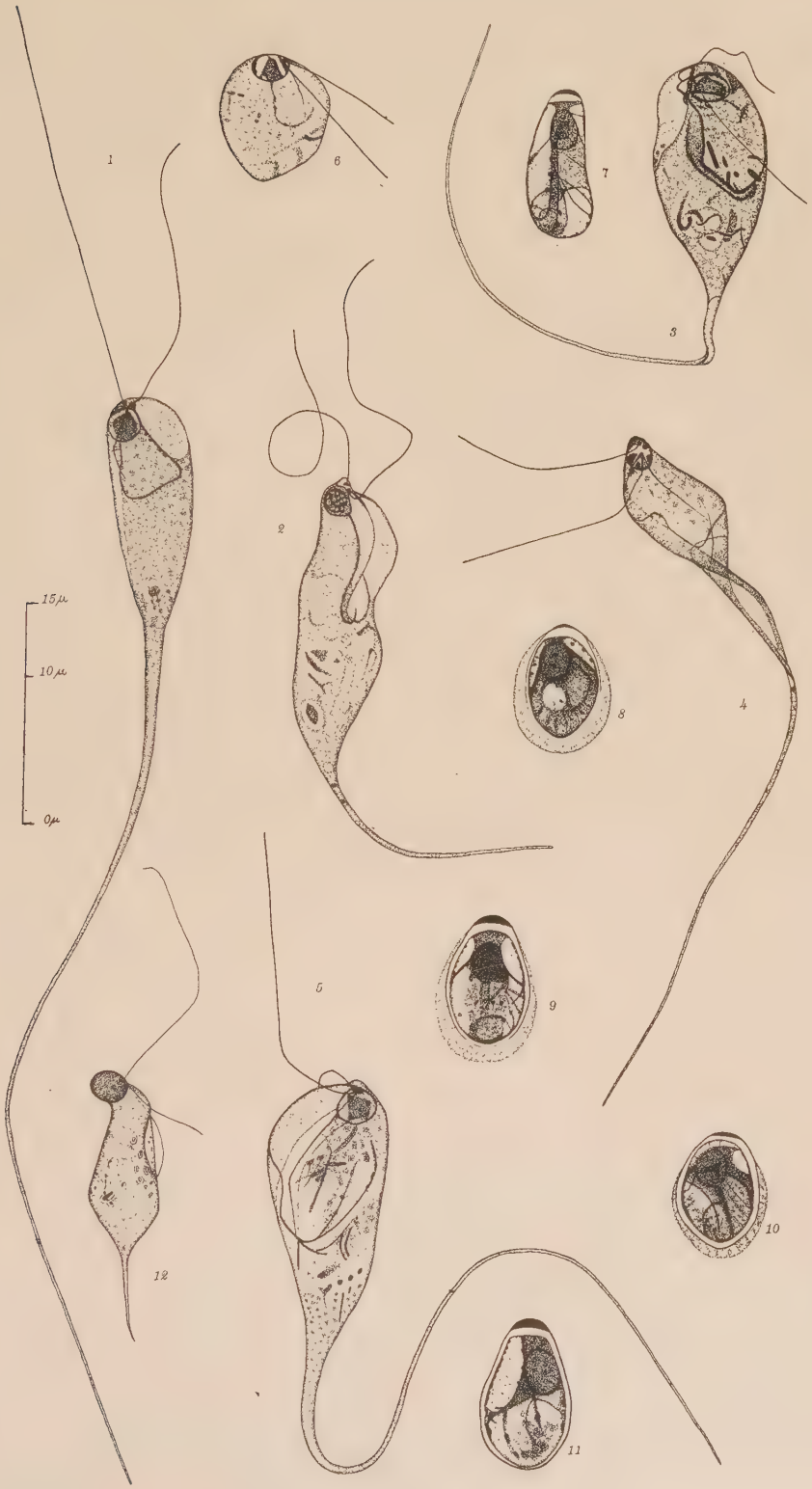
The nucleus, located very close to the anterior end, was quite like that described by Geiman (1932) for *R. caudacus,* from a gyrinid larva. There was frequently considerable stainable material on the nuclear membrane, and a cloud of stainable material occupied most of the center (Figs. 1–6).

The cysts.—These bodies ranged from 6.1 μ to 8.4 μ in length, averaging 7.3 μ. The width was from 4.6 μ to 6.1 μ, with an average of 5.1 μ. Roughly oval and tapering toward the anterior end, they showed a definite cyst wall. As noted by Hegner and Schumaker (1928) in *R. bradypi,* and by Hogue (1933, 1936) in several races of *R. intestinalis,* the cysts of *R. wenrichi* showed a thickened, deeply staining cap (Figs. 7, 8, 9, 10, 11). Even when not especially darkly stained, this greater thickness was in evidence. Unlike the situation as described by Hogue, in which the presence of acetic acid in the fixative prevents the appearance of the cap, those on *R. wenrichi* were quite prominent in the presence of this reagent.

The anteriorly located nucleus was darkly stained, and a tangle of fibers and threads extended posteriorly in the cysts. Anterior lighter areas, similar to those in the trophic stages, were common (Figs. 7, 8, 9, 10, 11).

A unique phenomenon apparently associated with the formation of the cyst wall was observed. In the most recently commenced encystment, the cap appears already established, but without much of the cyst wall (Fig. 7). Soon after this, a faintly staining layer appears about the whole animal (Fig. 8). The cyst wall now seems to be laid down in a progressive anterior-to-posterior fashion, on the innermost aspect of this layer (Fig. 8, wall about half way down; Fig. 9, wall complete except for the posterior region). This peculiar layer persists for a time after the cyst wall is complete (Fig. 10), but eventually disappears (Fig. 11).

FIG. 1. Very long specimen. Viewed from aboral surface.
FIG. 2. Typical retortamonad. Note apical peak, two basal granules, additional oral fiber accompanying the parabasal fiber, and the short course of the more posterior flagellum in the oral pouch.
FIG. 3. Note the thread accompanying the parabasal fiber, and the bent tail.
FIG. 4. Note spiral twist in body.
FIG. 5. Note apical peak, curved tail.
FIG. 6. Precystic form.
FIG. 7. Early cyst, with cap and no enveloping layer.
FIG. 8. Layer present, and cyst wall laid down in anterior region.
FIG. 9. Cyst wall nearly complete.
FIG. 10. Wall complete, with layer persisting.
FIG. 11. Wall complete, with layer gone.
FIG. 12. *R. caudacus,* redrawn from Geiman, 1932.



All figures are of *Retortamonas wenrichi*, n. sp., except number 12, which is *R. caudacus* redrawn from Geiman, 1932.

DISCUSSION

In general body form *R. wenrichi* more closely resembles Grassi's *R. gryllotalpae,* likewise from a mole cricket, and Geiman's *R. caudacus,* from a gyrinid larva, than any other species. The extreme length and the nature of the cysts, however, would be ample evidence for the creation of the new species. The formation of the peculiar layer about the encysting forms is a feature not heretofore described for these flagellates.

SUMMARY

1. A new species of *Retortamonas, R. wenrichi,* is described from an adult male common mole cricket, *Gryllotalpa hexadactyla,* collected in Ednor, Maryland.

2. Its most distinctive features are: (a) its extreme length (up to 76.0 μ), (b) the consistent presence of a thickened, more deeply staining cap on the cyst wall, and (c) the presence of a thin, lightly staining layer about the cystic animal, apparently associated with the formation of the cyst wall.

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EXOERYTHROCYTIC STAGES OF *PLASMODIUM DURAE*[1]

MYRON L. SIMPSON

In July, 1941, Herman reported the discovery of a new species of avian malaria, pathogenic to turkeys, and named it *Plasmodium durae.* In November, 1941, a duck infected with this species was secured and biological studies were started.

It proved impossible to secure turkey poults at the time the infected duck was obtained, therefore two 13-week-old turkeys were bought from a local breeder and were inoculated intravenously with 3 cc and 5cc, respectively, of blood, obtained by heart puncture, from the infected duck. It was felt that the rather large doses were necessary because of the age of the recipient animals. Nothing is known about the previous parasite history of these two turkeys, but blood examination at the time of their inoculation with *P. durae* was negative for parasites. Both turkeys were inoculated on November 14, 1941, and, in each case, parasites were first seen in blood samples eleven days later. Both birds continued to show parasites until their deaths.

Turkey No. 2 (Table 1) died after 26 days. This animal seemed to be partially paralyzed for several hours before its death, and although it was able to flap its wings, it could not raise its head or stand on its legs. An autopsy was performed and tissue smears made from the spleen, liver, lungs, and brain. These were stained with Giemsa. In smears from all of these organs, numerous bodies were found which were thought to be exoerythrocytic stages of *Plasmodium durae.* They were especially numerous in the lungs and brain, being present in the latter organ in such numbers as to occlude completely many of the capillaries. Some of these bodies were in a comparatively early stage of development, with only one or two nuclei, while many others were in later developmental stages, some apparently approaching segmentation. Other investigators in this laboratory who had had experience with exoerythrocytic stages, including Dr. Robert W. Hegner, Dr. Fruma Wolfson, and Miss Evaline West, were unanimous in the opinion that these bodies were unquestionably the exoerythrocytic stages of *Plasmodium durae.*

Turkey No. 1 (Table 1) was killed 78 days after inoculation; tissue smears of spleen, liver, lungs and brain were made and carefully examined, but no typical exoerthrocytic stages could be found in any of these organs.

Subsequently, as shown in the table, out of a total of 8 young (5–10 days old) turkeys which were inoculated intravenously with *P. durae,* two have been found to have exoerythrocytic stages in the spleen, liver, lungs and brain. Bird No. 4, which was 8 days old, was injected with 0.3 cc of inoculum from bird No. 1. The infected poult died 15 days later and tissue smears demonstrated the presence of exoerythrocytic stages in the usual sites. Bird No. 6, which was 10 days old, was injected with 0.8 cc of inoculum from a duck previously infected. This poult first showed parasites the next day, was patent for 10 days, remained latent for 5 days, then had a relapse and died 23 days after the initial infection. Tissue smears showed the presence of exoerythrocytic stages in the spleen, liver, lungs and brain.

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[1] From the Department of Parasitology, School of Hygiene and Public Health, Johns Hopkins University. The author is grateful to the late Dr. Robert W. Hegner for help in the early stages of the investigation, and to Dr. Fruma Wolfson for assistance in the preparation of this paper.

TABLE 1.—*Exoerythrocytic stages of* Plasmodium durae *in turkeys*

Bird No.	Age of bird	Size of inoculum, cc	Sub-patent period, days	Tissues examined					Survival after inoculation, days	Fate of bird
				Spleen	Liver	Lungs	Brain	Bone marrow		
1	13 wks.	3.0	11	−	−	−	−	*	78	Killed
2	13 wks.	5.0	11	+	+	+	+	*	26	Died
3	5–10 days	0.3	7	−	−	−	−	*	9	Died
4	5–10 days	0.3	5	+	+	+	+	*	15	Died
5	5–10 days	0.3	4	−	−	−	−	*	13	Died
6	5–10 days	0.8	1	+	+	+	+	*	23	Died
7	5–10 days	0.8	1	−	−	−	−	−	61	Killed
8	5–10 days	1.0	7	−	−	−	−	*	38	Died
9	5–10 days	0.7	7	−	−	−	−	−	50	Killed
10	5–10 days	0.6	7	−	−	−	−	−	50	Killed

* = smear of bone marrow not made.
+ = exoerythrocytic stages found.
− = exoerythrocytic stages not found.

Of the ten animals reported on, six died as a result of the experimental infections and four were killed at various intervals after the original infection. Exoerythrocytic stages were found in three of the six birds which died of the infection, but not in any of those which were killed. Smears of bone marrow were made in only three of the birds autopsied, but in none of them were the exoerythrocytic stages discovered.

In November, 1942, Purchase reported on a number of natural and experimental infections of *P. durae* in turkeys and other fowl. He stated that schizonts were frequently found in the endothelial cells lining the small blood vessels of the livers, spleens and brains of the experimentally infected birds. They were rarely seen in the lungs. It is of interest to note that in our birds we found the exoerythrocytic stages to be especially numerous in the lungs and brain, although it was not difficult to find them in considerable numbers also in the liver and spleen. It is quite possible that Purchase's "schizonts" and our "exoerythrocytic stages" are identical.

It should also be reported that at least 15 ducks, of various ages, infected intravenously with *P. durae,* were routinely studied in this laboratory. Since, as a general rule, the infection was not fatal to the ducks, they were killed at various intervals after infection, autopsied and tissue smears made. These also were stained with Giemsa and were carefully examined. However, in no instance have exoerythrocytic stages been demonstrable.

Of two old and eight young turkeys infected with *Plasmodium durae,* three were shown to have exoerythrocytic stages in the spleen, liver, lungs and brain. This evidence, while not exhaustive or complete, is believed to be sufficient to demonstrate that exoerythrocytic stages of this newly-discovered avian malaria parasite can and do occur.

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TRANSVERSOTREMA HAASI, A NEW FISH TREMATODE

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In a basin containing fish brought from the Red Sea about a dozen trematodes, which doubtlessly were parasites of one of these fishes, were found. As there were about twenty species of fish, it was not possible to determine the host. On investigation the specimens proved to belong to a new species of quite unusual structure, not fitting into any known genus or family. It is proposed to name it *Transversotrema haasi*.[1]

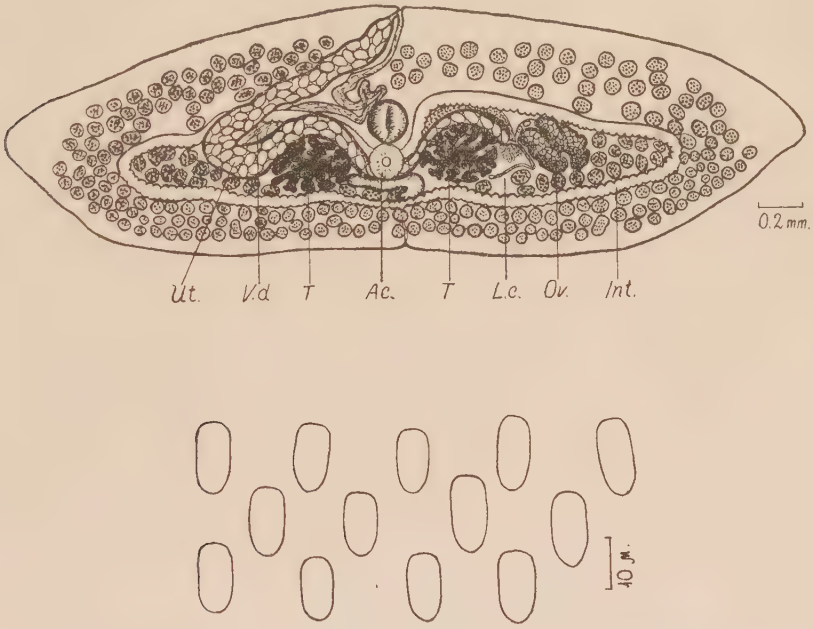

FIG. 1. (Top) Total view of *Transversotrema haasi*. Ac.—Acetabulum; Int.—Intestine; L.c.—Laurer's canal; Ov.—Ovary; T.—Testis; Ut.—Uterus; V.d.—Vas deferens. (Bottom) Body spines.

Description

Body flat, transversely elongated, lancet-shaped, length 0.8–1.25 mm, width 2.5–4.0 mm; ventral surface slightly concave, dorsal one slightly convex, both covered with short, stout spines disposed quicunctially; the spines are slightly larger, 15 μ, on the ventral surface than on the dorsal one. The oral opening has the shape of a longitudinal slit in the center of the body, opens into a globular pharynx, 0.2 mm in diameter; there is no oral sucker projecting above the surface of the body. Immediately behind the oral opening there is a flat disc-shaped acetabulum (not stainable *in toto* but plainly visible on sections) covered with a few concentric rows of minute spines. The oral opening leads to a narrow esophagus which divides into two intestinal branches. The latter bend forward, then towards the lateral extremities of the body and, before reaching them, bend again towards the center to unite between the oral opening and the posterior ridge of the body. Thus the intestine forms a closed loop running almost parallel to the edges of the body. The course of the intestine is rather smooth, but the contractions of the wall produce an almost con-

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[1] In honour of Dr. G. Haas, lecturer in Zoology, who kindly presented the material.

tinuous row of small diverticula. Two testes lie at the sides of the acetabulum, inside the intestinal circle. They occupy a globular space, 0.3–0.4 mm in diameter, but their structure is dendroid. Vasa efferentia unite just to the right of the right testis to form a voluminous, coiled, vas deferens which gradually becomes narrower and opens into the genital sinus in the middle of the anterior ridge of the body. There is no copulatory organ. The ovary consists of 2–3 big irregular lobes; it is a little smaller than the testes and lies between the left testis and the intestinal bend. A short funnel-shaped oviduct connects it with the oötype where it meets the Laurer's canal, uterus and yolk duct. The oötype is surrounded by a Mehlis' gland complex. The uterus has a transverse and an ascending part. The transverse part passes over the testes and behind the acetabulum, thus forming two waves. The uterus is more or less distended by numerous eggs, depending on the age of the worm; it opens into a common genital sinus. The yolk glands consist of numerous follicles which occupy almost the whole space free from other organs inside and outside the intestinal loop. Their secretion accumulates in a yolk sac lying between the first bend of the uterus and the first bend of the intestine. There is no seminal receptacle, its function is taken by the proximal part of the uterus which contains a mass of spermatozoa. The ova are 90 μ long and 70 μ wide; they have no filaments and contain a formed miracidium. The excretory vesicle is transversely elongated. The right extremity receives the excretory tubes from both sides of the body and the left one, widened and bent towards the middle line of the body, joins a narrow excretory duct which opens to the surface in the middle of the posterior edge of the body, just opposite the genital sinus.

The above described trematode does not correspond to any known genus and it does not fit into any known family. It is an aberrant form in every respect. In its asymmetrical transverse structure it resembles only one known species, *Moreauia mirabilis* Johnston, with which it has no taxonomic relation. In the absence of a copulatory apparatus and seminal receptacle and in parasitizing fish it recalls the family Mesometridae Poche. However, the circular intestine, the absence of the oral sucker, the presence of the acetabulum and of a peculiar excretory duct make the relation to this family doubtful. A new subfamily Transversotreminae is proposed for this genus.

ON A NEW GENUS AND SPECIES OF PARASITID MITE

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INTRODUCTION

In the course of experimental studies on the German cockroach, *Blattella germanica* (Linné), being carried on in this laboratory, 25 specimens of mites, representing a new species of the family *Parasitidae,* were found in cages housing the roach colony. As these specimens, 21 females and 4 males, differ from forms previously described, it seems advisable to erect a new genus and species for their proper classification. A new genus *Blattisocius* is proposed to include this mite, which, as *Blattisocius triodons,* is designated type species of the genus.

All specimens were killed in hot water, and mounted in a polyvinyl alcohol, lactic acid, phenol medium.

Genus Blattisocius gen. nov.

Color white. Movable chela bearing three teeth. Fixed chela about a third as long as movable chela, with a seta-like process near its tip. Anal plate bearing four paired and one unpaired setae, anteriorly truncate, larger than sternal plate. Peritremal and parapodal plates present. A pair of spindle-shaped plates present, one of which lies on either side of the dorsal plate between the latter and coxae I and II. A pair of large setae at posterior margin of dorsal shield.

Blattisocius triodons sp. nov.

Female: Color white, body longer than broad, shoulders not prominent. Legs II and III about equal in length. Legs I and IV about equal in length and longer than legs II and III. Tarsi of all legs bearing caruncles. Dorsal shield entire, not covering all of the dorsal surface. This shield bears about 32 pairs of setae, the most conspicuous being a very long pair at its posterior margin, and two pairs of small setae at its anterior margin (Fig 1). Sternal plate rectangular with two lateral projections near its anterior end. Extends from anterior margin of coxae I to posterior margin of coxae II. Slightly longer than wide. Bears three pairs of setae. Genito-ventral plate flask-shaped, posteriorly truncate. Extends from anterior margin of coxa III past posterior margin of coxa IV. Bears one pair of spines. Anal plate larger than sternal plate. Slightly more than two-thirds as broad as long. Anterior margin straight. Bears four paired setae and one unpaired seta (Fig. 2). Peritremal and parapodal plates present. Spiracles between coxae III and IV (Fig. 3 and 4). Small spindle-shaped plate on either side of dorsal plate, lying between dorsal plate and coxae I and II. Each plate connected with a peritremal plate by a single very narrow chitinous strand (Fig 3). Movable chela bearing three teeth, the most distal of which is largest. This chela is nearly straight throughout its length, slightly curved near tip, terminating in a point. Fixed chela about a third as long as movable chela, rod-like, a seta-like process near tip (Fig. 5). Palpi five-jointed and filariform. Labium with four pairs of setae. Chaetotaxy normal, dorsal and ventral surfaces of the opisthosoma possessing numerous setae. A pair of exceptionally large setae at the posterior margin of the dorsum.

Measurements of type specimen:

Total length (to anterior border of labium), 609 microns.

Body length (without capitulum), 504 microns.

Body width, 301 microns.

Leg I, 441 microns; Leg II, 301 microns; Leg III, 315 microns; Leg IV, 420 microns.

Male: The male has the same general characteristics as the female. The mouthparts show sexual differentiation as in males of many species (Fig. 6). Peritremal plate differs from that of female (Fig. 7). Dorsal shield as in female. Ventral plates two in number. Anterior plate extending from anterior margin of coxa I to posterior margin of coxa IV. Posterior plate broad, roughly triangular (Fig. 8).

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Fig. 1. Dorsal plate of female.
Fig. 2. Ventral plates of female.
Fig. 3. Peritremal plate of female, showing attached spindle-shaped plate, which lies dorsal to coxae I and II.
Fig. 4. Parapodal plate of female.
Fig. 5. Chelae of female.
Fig. 6. Chelae of male.
Fig. 7. Peritremal plate of male.
Fig. 8. Ventral plates of male.

Measurements of type specimen:
Total length, 410 microns.
Body length, 385 microns.
Body width, 217 microns.
Leg I, 322 microns; Leg II, 294 microns; Leg III, 287 microns; Leg IV, 329 microns.
Habitat: Debris in colony of German cockroaches, *Blattella germanica* (Linné).
Cotypes: Two females and one male deposited in the U. S. National Museum.

DISCUSSION

Only three mites were found on 238 roaches examined with the aid of a dissecting microscope. All other specimens were obtained from debris taken from the floor of the roach cages.

SUMMARY

Blattisocius triodons, representing a new genus and species of mite of the family Parasitidae, is described. A total of 25 specimens of this mite were taken from debris in cages housing a colony of German cockroaches, *Blattella germanica* (Linné). Of these specimens, 21 are females and four males. Only three mites were found on 238 roaches examined.

ACKNOWLEDGMENT

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PERICARDITIS IN *XENOPUS LAEVIS* CAUSED BY *DIPLOSTOMULUM XENOPI* SP. NOV., A LARVAL STRIGEID

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INTRODUCTION

The metacercariae of the strigeid trematodes usually occur, either as non-encysted or encysted forms, in the tissues of aquatic animals, particularly fishes and amphibians. Considerable damage to the tissues is produced and these infections often cause death (see Van Haitsma, 1931; Nigrelli, 1943). In these secondary hosts, practically every organ of the body may be attacked. Thus strigeid larvae have been found invading the skin, muscle, eyes, mesenteries, liver, kidney, spleen, gonads, brain and heart. Adult worms develop to sexual maturity in the intestine of reptiles, birds and mammals. The mature parasites also are capable of eliciting tissue reactions in some of the definitive hosts, causing such lesions as enteritis or tissue hyperplasia.

The strigeid larvae which produce pericarditis in *Xenopus* were previously reported by Southwell and Kirschner (1937). The present contribution deals with the pathology resulting from this infection, and a redescription of the fluke, together with notes concerning its taxonomy and probable life-history.

The writers wish to acknowledge the helpful suggestions and criticisms of Professor H. W. Stunkard, New York University.

PATHOLOGICAL PHYSIOLOGY

The larval flukes are found within the pericardial sac of South African toads and with the aid of a hand lens can be seen moving about in the fluid under the transparent pericardium (Fig. 1). The number of flukes present, particularly in toads that were dying, varied from 25 to 150. Other apparently healthy *Xenopus* invariably showed two or more of these parasites. Of 55 toads examined during the past two years, 43 were found to be parasitized, and those that died during the summer months were always heavily parasitized.

The parasite constitutes a foreign body, hence the protective response of the serous pericardial membrane is that of exudation. In the initial stages, the amount of fluid produced is small and therefore there is little if any effect on the heart. However, the continuous activity of many parasites, along with the possible toxic effects of their waste products, promotes the production of greater amounts of fluid. As the pericardial sac distends, it naturally encroaches upon other vital thoracic organs, especially the lungs. It is clear, therefore, that the lungs can no longer expand to their normal limits, and the result is a diminished vital capacity.

When the pericardial sac can distend no further, the intra-pericardial pressure rises until it reaches a point where it is greater than the pressure in the superior and inferior venae cavae. At this point a vicious cycle begins. Undue pressure on the

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sinus venosus, the superior and inferior venae cavae, and on the pre-caval veins interfere with the inflow of blood into the right auricle. This results in a diminished ventricular output with a corresponding drop in the systemic pressure, which in turn

Fig. 1. Dorsal view of the heart of *Xenopus laevis*. The striated flukes can be seen through the transparent pericardium. (Photograph by S. C. Dunton, N. Y. Zoological Society.)

results in slower oxygenation of the blood at the various respiratory organs, namely the lungs and skin. The anoxemia that results slowly paralyzes the respiratory centers and respiration ceases.

The clamping effect of abnormal pressure on the venae cavae, the sinus venosus, etc., tends to produce a congestion and subsequent stagnation of poorly oxygenated blood in the somatic tissues and viscera. This may account for the apparent vomiting reflex, which may be so powerful that an eversion and protrusion of the stomach may occur.

The efficiency of the heart is greatly diminished. The toads therefore die as a result of a true auto-toxemia resulting from a severe anoxemia. The affected heart

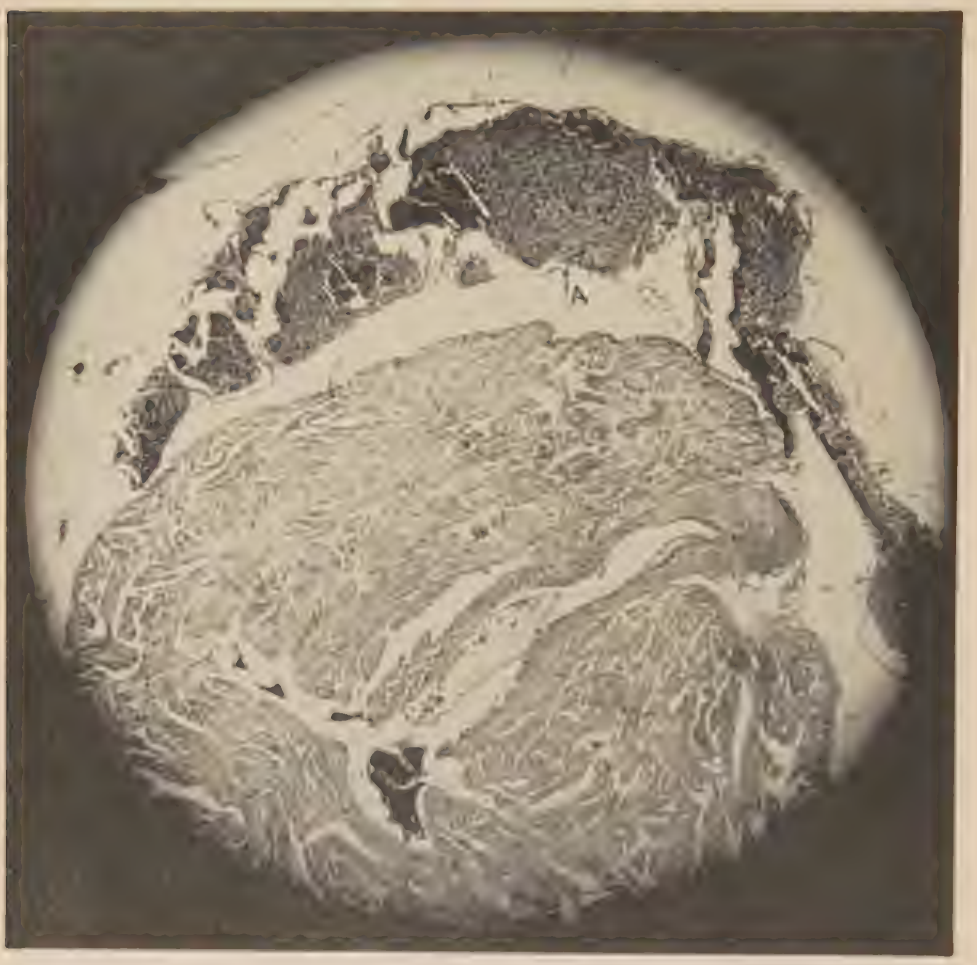

FIG. 2. Section through the ventricle of the heart, showing encapsulated semilunar haematoma (A) immediately below the epicardium. Intraventricular hemorrhages are evident within the myocardium, Haem.-Eosin. (Photograph by S. C. Dunton, N. Y. Zoological Society.)

continues to beat rhythmically after the respiratory centers no longer function, and finally it stops in systole, while the auricles continue to contract for some time.

Occasionally, as observed in several specimens, death is produced by haematoma-like formation within the ventricular wall (myocardium proper) or immediately under the epicardium (Fig. 2). Extravasation of blood into the pericardial sac, however, does not occur. This phenomenon may be explained by the fact that the fluid in the pericardial sac acts as a tampon on the bleeding points. In many

instances, extensive adhesions were present between the pericardium of the abnormally distended heart and the ventral thoracic wall.

DESCRIPTION OF THE PARASITE

The live strigeids of *Xenopus* are linguiform (Fig. 3). When examined under the microscope their movements are active, with the posterior end capable of expansion and contraction, much like the tail appendage in members of the Hemiuridae. However, this hindbody is not as well developed as in some other strigeids, although definitely demarcated from the forebody. When fully expanded, it is at least one-third the length of the forebody and cylindrical in form. If the hindbody is extended, the lateral margins of the forebody tend to fold ventrally. Such an infolding also occurs when the worms are freely suspended in the medium in which they are being observed. If the worms are in contact with the substratum, or if the hindbody is contracted, the flukes flatten out and the lateral folds disappear. In many of the fixed and stained specimens a slight infolding is found at the posterior lateral margins, particularly at the region where the fore and hindbodies meet.

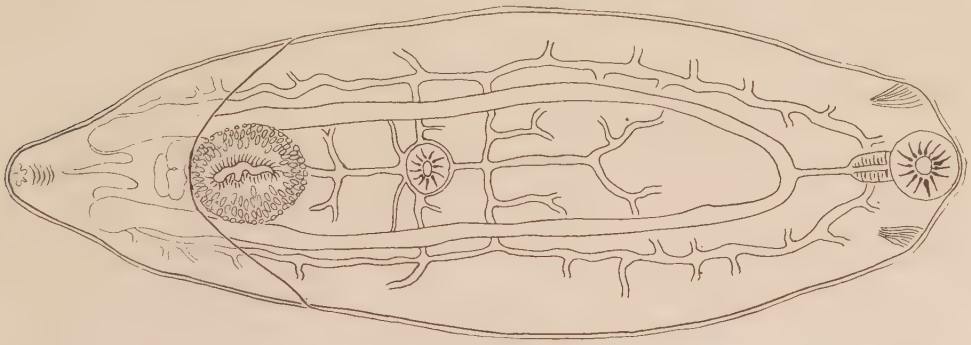

FIG. 3. Sketch of *Diplostomulum xenopi* made from a photomicrograph of a living worm. Details of tribocytic organ, cotylae, reserve excretory system, etc., added from observations on stained preparations. (× 125.)

The anterior end of the strigeid shows a slight lateral or auricular projection on either side of the oral sucker and these structures are especially evident in larger worms. The oral sucker is subterminal, well developed and followed by the pharynx. The oesophagus is relatively long and the intestinal crura pass to the posterior end of the body, deflect around the holdfast organ, terminating inside the hindbody. The lateral suckers or cotylae are poorly developed but possess both muscle fibers and gland cells. The acetabulum is comparatively small and oval in shape. It is centrally located in the anterior part of the posterior half of the forebody, some distance in front of the tribocytic or holdfast organ. The latter structure is large, oval in shape, the long axis corresponding to the long axis of the body. The cavity of the tribocytic organ is bordered by papillae and numerous proteolytic gland cells cover the dorsal surface. The primordia of the gonads are present just behind the tribocytic organ but within the hindbody. The excretory pore which is stellate in shape is found at the posterior tip, with branches of the reserve excretory system ramifying throughout the body. The system is typical of the strigeids. The main branches pass posteriorly from the region of the pharynx, and at the level of the anterior border of the acetabulum receive the branches coming from the intercecal region between the acetabu-

lum and the point of bifurcation of the ceca and between the acetabulum and tribocytic organ, and those branches coming from the extracecal areas of the posterior and anterior parts of the body. The main vesicles dilate and unite immediately before they reach the excretory pore. The body of the worms is filled with many calcareous concretions normally associated with such a reserve excretory system.

Specimens were fixed in warm bichloride of mercury with acetic acid and stained with paracarmine or Delafield's hematoxylin. In these preparations most of the concretions are dissolved. The flukes usually take on a more or less oval and flattened shape. The size of the worms varies considerably and many of the characteristics described above are obscured. For matter of record the following measurements of fixed and stained worms are given: Size of the body varies from 0.2×0.25 to 0.76×0.31 mm (av. 0.52×0.3 mm); oral sucker, 0.04 mm; pharynx, 0.017×0.008 mm; acetabulum, 0.038×0.045 mm; holdfast organ, 0.11×0.17 mm; distance between acetabulum and tribocytic organ varies from 0.17 to 0.33 mm. In these stained specimens, the oesophagus, intestinal crura, reserve excretory branches and limits of the hindbody become indistinct for detailed study. The cuticle is thin and without spines. It should be pointed out that the description and measurements given here differ on many points from those reported originally by Southwell and Kirschner (1937).

CLASSIFICATION OF STRIGEIDS FROM *Xenopus*

The strigeids of the clawed toad are definitely diplostomes. They were found in the pericardial sac immediately on arrival of the anurans in this country from South Africa, and they were continuously recovered from toads that died during a period of more than two years after their importation.[1] This indicates that the flukes never encyst and the infection may persist for as long as the toads remain alive. The fact that worms of varying sizes were found in the pericardial fluid demonstrates that the toads were being continually reinfected while in their native habitat. Examination of other organs and tissues of the body revealed no parasites or evidence which would indicate the migration path taken by these flukes, and further shows that by the time the toads arrived in this country the worms had already reached their final location in the host.

Other strigeids have been reported from the heart region of various hosts. Many species of the group Tetracotyle are invariably found in thick cysts in the pericardium of marine and fresh-water fishes (see Van Cleave and Mueller, 1934; Nigrelli, 1943). Odlaug (1940) reported the mesocercariae of *Alaria intermedia* as occurring free, among other tissues and organs, in the pericardial region of tadpoles, and frogs (*Rana pipiens*) less than a year old. The metacercariae and adults of *A. intermedia* are found in mammals. A similar four-host cycle was reported by Bosma (1934) for *Alaria mustelae* in which the mesocercarial forms are found in the tissues of frogs (*Rana catesbiana* and *R. clamitans*) and metacercariae in mammals.

The presence of lateral suckers and primordia of the gonads shows that the strigeids of the clawed toad are metacercarial and not mesocercarial forms. The fact that these worms are easily digested at room (ca. 25° C) and incubator temperatures

[1] These anurans are imported for use in the biological assay of sex hormones and determination of human pregnancy (See Weisman, Snyder and Coates, 1942).

(37° C) when exposed to artificial ferments[2] would suggest that final host may be some reptilian form.

In 1936, Dubois erected the family PROTERODIPLOSTOMIDAE to include the strigeids parasitic in reptiles. The family is a fairly homogeneous group and is characterized mainly by the position and arrangement of the reproductive systems and sub-divided on the basis of size and shape of the tribocytic organ, the presence or absence of papillae on this organ and differences in the distribution of the vitellaria. With the exception of *Proalaroides serpentis* Yamaguti (1933), the forebody of the flukes are more or less flattened and all lack the lateral suckers. There are striking similarities between the strigeids of *Xenopus* and the members of the PROTERODIPLOSTOMIDAE Dubois, 1936. Except for the presence of the lateral suckers, the worms are closely similar to certain POLYCOTYLINAE Monticelli, 1888, such as *Crocodilicola* Poche, 1925, and *Pseudocrocodilicola* Byrd and Reiber (1942). It is with difficulty that the lateral suckers of the strigeids of the clawed toad can be demonstrated but there can be no doubt that the cotylae are present. The question arises, because of this fact, whether or not these lateral suckers have been overlooked by other investigators or whether or not they disappear as the worms reach maturity. It is unfortunate that the life-history is not known for the typical members of the family. But it may be suspected that the larval forms show up in amphibians. This is known to be the case for *Proalaroides serpentis* Yamaguti, a proterodiplostomid (OPHIODIPLOSTOMINAE Dubois, 1936) which occurs in the intestine of the cobra, *Elaphe quadrivirgata,* and the metacercariae of which are found encysted in the musculature of the frog, *Rana nigromaculata. Proalaroides serpentis* has well developed cotylae, but the size and shape of the body and the tribocytic organ together with other organ relationships eliminates this genus as the probable adult of the strigeids of *Xenopus.*

Southwell and Kirschner (1937) concluded that the strigeids from the clawed toad were identical with *Tylodelphys rachiaea* (Henle, 1833), a larval form redescribed by Lühe (1909) from the rachidian canal of the European frog (*Rana temporaria*). Lühe suggested that the adult form may be *Hemistomum excavatum* (Rud., 1803). This assumption was shown to be correct when Ciurea (1928) experimentally demonstrated that the larval *T. rachiaea* from frogs developed into *Proalaria excavata* (Rud.) in the small intestine of the stork, *Ciconia ciconia.* This part of the cycle was confirmed by Szidat (1935) and the life history completed by his discovery of the forked-tailed cercarial stage in *Planorbis corneus* (L.). It should be pointed out that *Tylodelphys rachiaea* (larval forms), *Hemistomum excavatum* and *Proalaria excavata* are considered by Dubois (1938) to be synonyms of *Tylodelphys excavata* (Rud.). All the described species of *Tylodelphys* occur in birds, and the metacercariae are found in fishes or amphibians.

The chief characteristics of the genus *Tylodelphys* are: a distinct trilobed appearance of the anterior end, weakly developed but nevertheless distinct lateral suckers, presence of a prepharynx, a relatively short esophagus and comparatively large tribocytic organ. The strigeids from the African clawed toad have weakly developed but indistinct lateral suckers, no prepharynx, relatively long esophagus and medium sized tribocytic organ possessing papillae. Such characteristics certainly eliminate these strigeids from the genus *Tylodelphys.* However, on the basis of distinct differ-

[2] 1 gr. of pepsin in 100 cc of N/0.01 HCl; 50–50 mixture of 1% pancreatin and 0.5% sodium bicarbonate.

ences in organ relationships, size, shape and structure of the tribocytic organ, distribution of the proteolytic gland cells, degree of development of the lateral suckers, site of infestation, differences in host and geographical location of the host, we consider the strigeids from *Xenopus* to be different from those previously described from other amphibians and for these reasons they are tentatively identified as *Diplostomulum xenopi,* sp. nov.

SUMMARY

The pathological physiology of the heart of *Xenopus laevis* resulting from strigeid fluke infection is described.

The parasite producing the pericarditis and other lesions of the heart is redescribed and considered to be a new species for which the name *Diplostomulum xenopi* is proposed.

The taxonomic relationships are discussed and probable life-history suggested.

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CERCARIA SOLEMYAE N. SP., PROBABLY A BLOOD FLUKE, FROM THE MARINE PELECYPOD, *SOLEMYA VELUM*

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In the summer of 1937, during the course of a survey of trematode infections in molluscs, some cercariae were obtained from the bivalve, *Solemya velum,* collected in the vicinity of Woods Hole, Massachusetts. During subsequent summers, similarly infected *Solemya velum* have been collected but never in great numbers. The anatomy of these cercariae strongly suggests a close relationship to the blood-parasitizing trematodes.

MATERIAL AND METHODS

Specimens of *Solemya velum* were placed in finger bowls of sea water and observed daily for the emergence of cercariae. Live cercariae, fixed and stained whole mounts, and serial sections, were studied.

SPOROCYST

(Fig. 6.)

Cercaria solemyae n. sp., develops in small, oval or spherical sporocysts in the gonad and digestive gland of the bivalve mollusc, *Solemya velum.* Some infections are extremely heavy and must result in disturbances of digestion and reproduction of the host. Fig. 7 shows a severe infection. A daughter sporocyst generation is present. Few cercariae are contained in a sporocyst, not more than five were observed in any one. The wall of the sporocyst is thin except at one end where a knob of cells occurs.

The incidence of infection seems to vary with different collecting grounds. From some regions no infected individuals could be found. Sixty-three *Solemya velum* were collected from one locality and all were negative. From other localities the infection rate was as high as 9 per cent.

CERCARIA

(Figs. 1–5)

The body of the cercaria is small, measuring (living material, three specimens) from 0.089 mm to 0.098 mm, averaging 0.094 mm in length by 0.025 mm to 0.030 mm, averaging 0.027 mm in width. Measurements of fixed material (ten specimens) range from 0.059 mm to 0.078 mm with an average of 0.067 mm in body length by 0.022 mm to 0.034 mm with an average of 0.026 mm in body width. The sides of the body present a crenated appearance partly due to short rows of relatively large spines. There are about twenty-eight rows of these spines on each side of the body, each row including three or four spines. These spines are about three microns in length. In addition to these, there are longer, bristle-like spines on each side of the body, some near the exits of the cephalic gland ducts and others located a distance of about one-fifth the body length from the posterior end. No suckers are present. The mouth is subterminal and has an irregular outline which varies somewhat as shown in Figs. 2 and 3. The digestive tract consists of a slender tube leading from the mouth to the pouched gut near the middle of the body. The gut has five pouches in most specimens but occasionally only four. The cephalic glands are five in number on each side of the body. The gland cells are somewhat laterally arranged and occupy the middle two-fourths of the body. They stain deeply and quickly with

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neutral red. Their ducts pass anteriad in a group and empty into two pit-like depressions at the anterior end of the body (Fig. 4). The distal ends of the ducts may be swollen and are arranged in a circle so that in cross section they form a rosette (see Fig. 5). The excretory system has been partially traced. The excretory bladder is divided into three parts, two small, lateral sacculations and a middle larger one. The entire bladder measures about 0.0036 mm long by 0.0058 mm wide. The lower ends of the main collecting ducts were seen but could not be traced cephalad. Two minute flame cells were seen on each side of the body but it is highly probable that there are more.

The tail is very short and is capable of only limited change in size and shape from almost spherical to pyriform. Tail measurements of living material ranged from 0.016 mm to 0.019 mm with an average of 0.017 mm in length by from 0.011 mm to 0.014 mm with an average of 0.0125 mm in width. The surface of the tail is crenated like the body. There are two bristles that project outward, one from each side of the tail. An excretory tube extends from the bladder into the tail where it divides into two blind sacs. The tail has practically no function as an organ of locomotion. In fact, the cercaria as a whole moves very little.

Cercaria solemyae is most like the cercariae and adults of the family APOROCOTYLIDAE. It resembles these forms in the absence of suckers and in the possession of groups of spines laterally arranged [similar to *Sanguinicola armata* and *S. intermedia,* Ejsmont (1925), and *Paradeontacylix sanguinicoloides* McIntosh (1934)]; a gut provided with pouches [similar to the cercaria of *Sanguinicola* Odhner (1911), adult of *Paradeontacylix sanguinicoloides* McIntosh (1934), and adult of *Deontacylix ovalis* Linton (1910)]; and an irregular mouth outline [similar to *Sanguinicola armata* Ejsmont (1925)]. It differs from all known blood-fluke cercariae in the nature of the tail, since all the others have forked tails.

There are, of course, pitfalls in anatomical similarities but several characters of *Cercaria solemyae* are so strikingly similar to certain blood fluke cercariae or adults, particularly those parasitizing fishes, that it is believed that this form is a new type of blood fluke cercaria. Some attempt has been made to complete the life cycle but due to lack of time it was not successful. It is hoped that after the war, this work may be carried on to a satisfactory conclusion.

EXPLANATION OF PLATES

ABBREVIATIONS USED

C—cephalic gland
Ce—cercaria
D—duct of cephalic gland
E—excretory bladder
F—flame cell
G—germ ball
I—intestine
M—mouth
T—excretory tube in tail

PLATE I

FIG. 1. Camera lucida drawing of *Cercaria solemyae* n. sp.

FIGS. 2 and 3. Outlines of the shape of the mouth.

FIG. 4. One of the pits at the anterior end of the cercaria into which the cephalic glands empty.

FIG. 5. Cross section of the swollen portions of the cephalic gland ducts showing their rosette arrangement.

FIG. 6. A sporocyst containing two cercariae and a germ ball.

PLATE II

FIG. 7. Longitudinal section through the body of the clam, *Solemya velum,* showing the parasitized gonad (1), digestive organ (2) and a ganglion of the host (3).

FIG. 8. Section adjacent to the one shown in FIG. 7, but under higher magnification. Below the host ganglion (3) are seen parasitized gonad tubes.

FIG. 9. Some of the parasitized gonad tubes under still higher magnification (4) indicates a cercaria within a sporocyst.

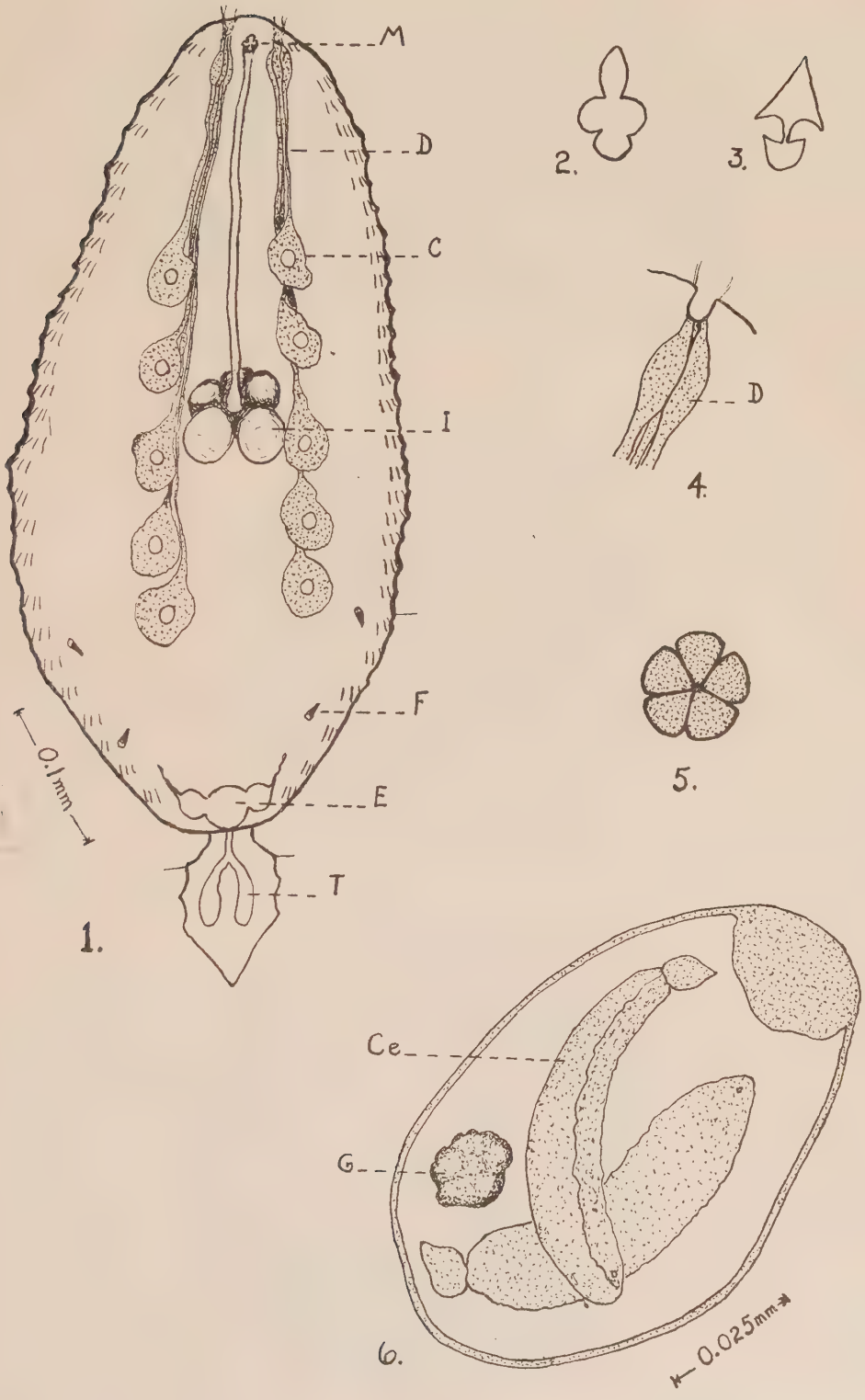
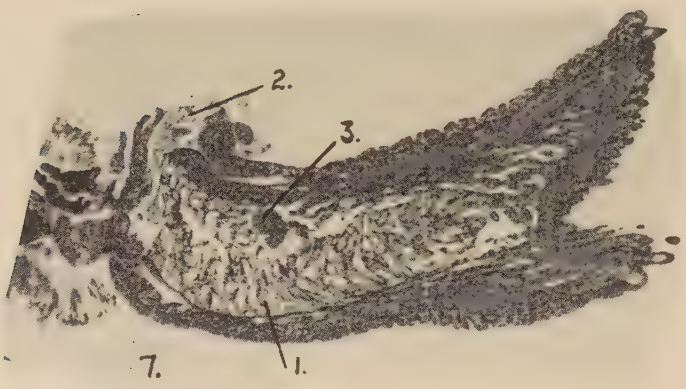
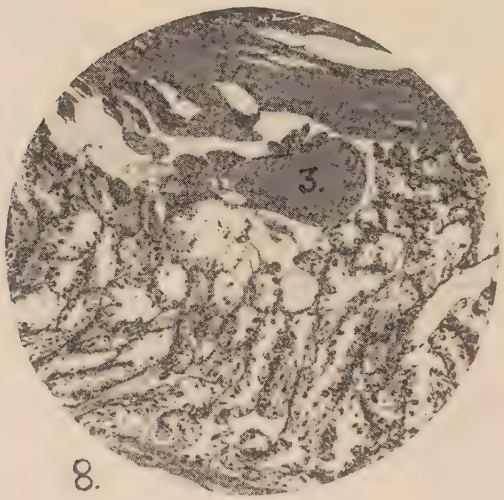


PLATE I





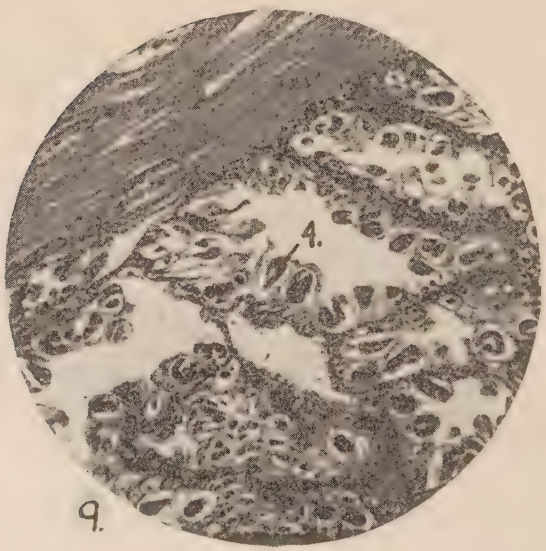


PLATE II

SUMMARY

A new species of larval trematode, *Cercaria solemyae,* developing in sporocysts in the pelecypod, *Solemya velum,* is described.

The anatomy of the body of this cercaria strongly suggests that it is one of the blood flukes.

The tail of this cercaria is very small and is not forked, thus differing from all known blood fluke cercariae.

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WAR-TIME ACTIVITIES OF MEMBERS OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

HENRY E. MELENEY*

In my presidential address for 1942,[1] I outlined some of the ways in which members of the American Society of Parasitologists might contribute to the winning of the war. In December 1943 it was decided to determine, as far as possible, the current war-time activities of the members of the Society. Dr. James T. Culbertson, Secretary of the Society, sent return postcards to the 426 members in good standing asking for information with reference to their present employment and activities. After most of these cards were returned, it was decided to send a questionnaire to the 101 members who were delinquent in their dues, in view of the fact that some of them were undoubtedly located where they could not keep up their active membership.

As a result of this survey, information concerning 442 (84 per cent) of the members of the Society has been received and is presented in Table 1. It will be noted that 80 members of the Society were in military service, 36 of these being already overseas when the cards were sent in. It is probable that a considerable number of the members from whom replies were not received are also in military service overseas and did not have an opportunity to reply. Accurate data would probably indicate that about 20 per cent of the membership is in military service.

Among the 69 members employed by civilian governmental services the large majority are undoubtedly occupied in research or administrative positions involving war activity. Two of these are known to be in overseas service.

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[1] Meleney, H. E. 1943. The Role of Parasitologists in World War II. Am. J. Parasitol. 29: 1–7.

TABLE 1.—*War-time activities of 442 members of the American Society of Parasitologists as of December 1943–April 1944*

Military Service, 80.	
Army, 62.	
Continental United States	33
Overseas	29
Navy, 18.	
Continental United States	11
Overseas	7
Civilian Governmental Services, 69.	
U. S. Public Health Service, 13.	
Continental United States	12
Overseas	1
U. S. Department of Agriculture, 28.	
Bureau of Animal Industry	17
Bureau of Entomology	7
Other	4
Other Federal Agencies, 8.	
Continental United States	7
Overseas	1
State Governmental Agencies	17
Local Governmental Agencies	3
Civilian Teaching Positions, 185.	
Universities, Colleges, High Schools	117
Medical, Dental, Veterinary, Public Health Schools	68
Private Research Institutes	3
Rockefeller Foundation	9
Medical Students	10
Commercial Firms, 9.	
Pharmaceutical	4
Other	5
Hospitals and Medical Laboratories	7
Puerto Rican Members	4
Retired	9
Miscellaneous	8
Foreign Members in Allied Countries	34
Members in Enemy Occupied Territory, 15.	
Philippines	5
China	4
Japan	3
Germany	2
Belgium	1
Total	442

The largest group of members, 185, is engaged in teaching and research in civilian educational institutions. More than one-third of these are in schools related to medicine and are, therefore, directly occupied in teaching students who are already in military service. Undoubtedly, many of those in other universities and colleges are teaching students who are in military service. In addition, a considerable number in both medical and non-medical institutions are engaged in goverment research projects or in research directly or indirectly connected with the war effort.

Among the other groups, it will be noted that there are 10 medical students, and that institutions or agencies are represented which are devoting their major effort to war activities.

The Puerto Rican members are listed separately to indicate that they are not in the continental United States, although they are not to be included among the foreign members in allied countries.

Finally, particular attention should be called to the 16 members of the Society in enemy occupied territory. Those in the Philippines and China consist of natives of those countries who are under the domination of the Japanese. The single member in Belgium is also under enemy domination. The three members in Japan and the two in Germany are natives of those countries.

This brief summary indicates that the American Society of Parasitologists is taking an important part in the war effort. It is probable that before the war is over more of the members will enter military service and others will undoubtedly undertake research projects under government auspices. The tremendously increased attention which is being given to human parasitology as a result of the war will undoubtedly create a permanent interest in this field which will be reflected in a greater demand for the teaching of this subject in the curriculum of medical schools and for the solution of research problems.

RESEARCH NOTES

THE EFFECT OF PRICKLY-ASH BARK UPON THE EFFICACY OF PHENOTHIAZINE AGAINST NEMATODES IN FOWLS

Phenothiazine has proved itself an anthelmintic of unusual merit although it possesses certain prominent disadvantages. For example the dose for some animals is bulky, the drug is somewhat expensive, and it may be toxic when used in some animals. An effective synergist might obviate in part some of these objections. The present report is an unsuccessful attempt to discover such a synergist, and it is recorded largely to direct attention to an unexplored field of phenothiazine research.

Haller, La Forge, and Sullivan (1942, J. Econ. Entomol. **35**: 247–248) reported that sesamin and sesame oil, as well as asarinin which is found in the bark of southern prickly ash, activate the insecticidal properties of pyrethrum. Since the pure chemicals are not available some bark of southern prickly ash, *Xanthoxylum Clavo-Herculis,* was obtained. One gram of bark plus 0.2 gram of phenothiazine was given to each of 6 Ancona cockerels. Six similar cockerels, receiving 0.2 grams of phenothiazine alone, served as controls. Results are presented in Table 1. The

TABLE 1.—*Comparative efficacy of 0.2 grams of phenothiazine and of 0.2 grams of phenothiazine mixed with 1 gram of* Xanthoxylum Clavo-Herculis *bark for the removal of* Ascaridia *and* Heterakis *from chickens**

Band No.	Weight at time of treatment	Worms removed by treatment		Worms found at necropsy		Per cent	
		Ascaridia	Heterakis	Ascaridia	Heterakis	Ascaridia	Heterakis
Phenothiazine mixed with bark of *Xanthoxylum Clavo-Herculis*							
	Grams						
3596	1715	0	5	8	0	0.0	100.0
3592	1505	0	129	32	1	0.0	99.2
3591	1560	0	88	59	0	0.0	100.0
3595	1860	0	25	1	11	0.0	69.4
3958	1540	10	16	25	0	28.6	100.0
3597	1600	0	6	1	0	0.0	100.0
Total		10	269	126	12		
Phenothiazine alone							
3662	1560	0	80	6	0	0.0	100.0
3663	1530	0	10	6	0	0.0	100.0
3671	2070	0	17	5	1	0.0	94.4
3665	1780	46	147	102	252	31.1	36.8
3666	1515	0	71	0	0	...	100.0
3667	2000	23	67	9	0	71.9	100.0
Total		69	392	128	253		

* These Ancona cockerels were dosed January 10, 1943, and necropsied 3 days later.

mixture of phenothiazine with bark of *X. Clavo-Herculis,* removed 7.3 per cent of 136 *Ascaridia* and 95.7 per cent of 281 *Heterakis;* the phenothiazine alone removed 35 per cent of 197 *Ascardia* and 60.7 per cent of 645 *Heterakis.* The total figures alone suggest that bark of *X. Clavo-Herculis* may have interfered with the activity of phenothiazine against *Ascaridia* and stimulated activity against *Heterakis.* However, the records of the individual birds suggest that the addition of bark was without effect since the differences in the percentages of worms removed are largely due in each case to the record of a very few heavily infected birds. We conclude, therefore, that 1 gram of the bark of *Xanthoxylum Clavo-Herculis* did not influence appreciably the efficacy of a dose of phenothiazine administered simultaneously.—PAUL D. HARWOOD AND JAMES E. GUTHRIE, *Dr. Hess and Clark, Inc., Ashland, Ohio.*

NOTES ON THE FEEDING OF CONE-NOSED BUGS (HEMIPTERA, REDUVIIDAE)

Osimani (1942, J. Parasitol. **28**: 147–154) studying some parasites of reptiles in South America fed *Triatoma rubrovaria,* a blood-sucking bug, upon the lizard, *Tupinambis teguixin,* after noting that Talice found these bugs in the burrows of this lizard. For the past few years, although many adults of *Triatoma longipes* have been collected at the Alvarado Mine in Arizona, a careful search of wood rat houses did not reveal this site as a breeding ground for this species of bug (Wood, 1941, Southwestern Med., April, 112–114). Possibly the nymphs of this Reduviid vector of Chagas' disease in the United States feed on reptiles in underground burrows as indicated by the following notes.

Triatoma longipes Barber. On May 18, 1942, during a period of relatively warm weather, the writer observed 3 small nymphs attempting to feed on the tail (1) and leg (2) of one western gecko, *Coleonyx variegatus.* Twenty-four hours later 4 of 20 small nymphs had fed to capacity in a jar with 5 adult geckos. On the same day, 26 starved small nymphs were put into a 10-inch Petri dish with 2 western worm snakes, *Leptotyphlops humilis humilis.* On May 20, 1942, these bugs were removed. One nymph was dead and 5 small nymphs fed on the snakes, two to capacity as evidenced by their well-rounded, dark-red abdomens. The remaining 20 apparently did not feed, since their abdomens were still flat in cross-section. These nymphs were placed in a jar with 1 adult ♂ desert scaly lizard, *Sceloporus magister magister.* After a 24-hour exposure, 4 were dead, 12 had been eaten by the lizard, and one of the remaining 4 live nymphs had fed to capacity on the lizard.

On May 20, 1942, a very warm day, 4 of 10 small *longipes* nymphs placed in a jar over a 24-hour period with 1 immature desert crested lizard, *Dipsosaurus dorsalis dorsalis,* fed on this lizard, some of them feeding to capacity. On the same day, 4 of 5 small nymphs fed on 1 adult western collared lizard, *Crotaphytus collaris baileyi;* 3 of 5 fed on 1 adult desert side-blotch lizard, *Uta stansburiana stejnegeri;* and 5 of 5 fed (three to capacity) on 1 desert leaf-nosed snake, *Phyllorhynchus decurtatus perkinsi,* each group over a 24-hour period. On the same day, 5 of 10 small nymphs fed (3 to capacity) on 2 immature yucca night lizards, *Xantusia vigilis,* resulting in the death of 1 lizard. Ten small nymphs placed in a jar with 2 adult western geckos, *Coleonyx variegatus,* were eaten by the lizards over a 24-hour period but the presence of blood spots on the sides of the jar (from crushed bugs) indicated that some of the bugs had fed on the geckos before being eaten.

On May 21, 1942, 35 small *longipes* nymphs, 18 of which were starved, i.e., with flat abdomens, were placed in a cage with 7 adult yucca night lizards, *Xantusia vigilis.* Nine of these had fed on the lizards by May 24th. On May 22, 1942, 2 of 11 small nymphs fed to capacity on 1 adult southern California alligator lizard, *Gerrhonotus multi-carinatus webbii;* 8 of 10 fed on 1 adult western glossy snake, *Arizona elegans occidentalis;* and 9 of 10 fed (6 to capacity) on 1 adult red racer, *Coluber flagellum frenatum,* each group feeding over a 48-hour period.

On June 23, 1942, 3 small nymphs of *Triatoma longipes* were placed in a jar at 3:00 PM with 1 Jerusalem cricket, *Stenopelmatus longispina* Brunner. By 3:50 PM, 1 nymph had fed on the cricket and 1 nymph was eaten by it. The *Triatoma* fed through the conjunctival membrane of the lateral abdominal wall. By 8:00 AM of June 24th, both of the remaining bugs were eaten by the cricket.

Thus, it appears that *Triatoma longipes* is not entirely dependent upon Arizona warm-blooded animals, especially mammals, for its food supply but may feed on reptiles and other cold-blooded vertebrates as Brumpt (1927, Presse Méd. 35: 1161–1162) reports for *Triatoma rubrovaria* from Uruguay.

Triatoma rubida (Uhler). On May 21, 1942, 3 of 5 small nymphs fed (2 to capacity) on 1 adult ♂ desert scaly lizard; 3 fed to capacity on 1 adult ♂ desert side-blotch lizard; 4 fed (2 to capacity) on 1 adult western collared lizard; and 2 fed on 1 desert leaf-nosed snake, each group within a 24-hour period. On May 25, 1942, 3 medium nymphs fed (2 to capacity) on 1 adult western gecko. On May 26, 1942, 2 ♂ *rubida* failed to feed on 1 western glossy snake, and 2 other ♂'s did not feed on 1 red racer, each over a 24-hour period.

Triatoma protracta (Uhler). On May 24. 1942, 3 medium nymphs fed (2 to capacity) on 1 adult ♀ desert side-blotch lizard; 1 did not feed on 1 ♂ desert scaly lizard; 1 fed to capacity on 1 immature desert crested lizard; 4 of 5 fed to capacity on 1 adult western collared lizard; 5 fed (3 to capacity) on 1 adult western glossy snake; and 5 fed (4 to capacity) on 1 adult red racer, each within a 24-hour period. Two medium nymphs were observed to feed immediately on 1 adult southern California alligator lizard.

The site of feeding when noted was the fine-scaled portion of the lizard's body, as side of neck, armpit, inguinal area or under body surface. Bugs feeding on the coarse-scaled portion of the lizard's body would apparently feed through the skin between the larger scales. Whether *Triatoma protracta, T. rubida,* and *T. longipes* feed in nature on cold-blooded vertebrates can be determined by examining the stomach fluids of recently captured bugs for nucleated red cells of characteristic size, shape and structure.—SHERWIN F. WOOD, *1015 No. Alexandria Ave., Hollywood 27, California.*

PARAGLAUCOMA SP., A FACULTATIVE PARASITE OF COELENTERATES

Several specimens of *Hydra americana* collected from a pond on the University of Pennsylvania campus, examined during the spring of 1943, contained within their gastro-vascular cavities numerous active ciliates meeting in all details the morphologic criteria of the genus *Para-*

glaucoma. According to Kudo (1939, Protozoology, p. 550) this genus comprises only one representative species, *P. rostrata* Kahl, living in fresh water and often found in dead rotifers. Kahl (1926, Arch. f. Protistenk, **55**: 351–353) reports finding them in a patch of moist moss and free living.

The organism present in *Hydra* did no serious damage, although on several occasions the ciliates were seen to escape from the digestive cavity to the external medium by forcing their way through the body wall of the host. The question of infection was somewhat complicated by the observation that the same ciliate occurred in the bodies of Daphnia which served as food for the coelenterates. They were, however, not seen in living Daphnia, so that the involvement of two or more hosts belonging to different phyla is entirely a matter of speculation. If *Paraglaucoma* is normally a scavenger, subsisting on decaying animal matter in fresh water, its complete immunity to *Hydra* digestive enzymes strongly suggests facultative parasitism. The ciliate was never encountered in *Chlorohydra viridissima*. The latter species was collected from the same source and kept in the same culture dishes with the infected *Hydra americana*.

During the summer of 1943 an organism strongly resembling the above *Paraglaucoma* was frequently found on and in living colonies of *Campanularia flexuosa*, gathered from the Provincetown breakwater. This parasite definitely did not enter the hydroid colonies with the food (via a primary host), but actively penetrated into mature gonangia or into injured portions of the hydrocaulus, where the protective perisarc was lacking. It never entered any hydranths through the manubrium, as it was not immune to the action of nematocysts. Aging hydranths which had lost their tentacles were attacked by the ciliate. Planulae of *Campanularia* kept for observation in Syracuse watch glasses were likewise attacked and destroyed. The parasite showed considerable size differences (60–110 μ long) and variation in opaqueness depending on the amount of food ingested.

Substances, probably enzymatic, secreted by the parasite had a remarkable cytolysing effect on the hydroid coenosarc. Within one hour this ciliate cytolyses from 30 to 40 times its own volume of host tissue, which demonstrates its potentially devastating inroads upon hydroid communities.

The aim of this note is not to suggest the establishment of a new species for the above two organisms, but to draw attention to the wide range of habitat (moss, pond water, hydra, marine hydroids, dead rotifers and dead daphnia) and the versatility of food habits of representatives of the genus *Paraglaucoma*. In its ecology *Paraglaucoma*, therefore, resembles *Glaucoma* which has been reported from an astonishing variety of potential hosts (1941, G. N. Calkins and F. M. Summers, Protozoa in Biological Research, New York, article by H. Kirby, Jr., pp. 894–899).—T. S. Hauschka and R. B. Doll, *University of Pennsylvania and Marine Experimental Station of the Lankenau Hospital Research Institute, North Truro, Mass.*

AN ADDITIONAL CALIFORNIA LOCALITY FOR *TRYPANOSOMA CRUZI* CHAGAS IN THE WESTERN CONE-NOSED BUG, *TRIATOMA PROTRACTA* (UHLER)

The writer, with the aid of members of United States Navy Malaria and Epidemic Control Team #40, collected 188 *Triatoma protracta* from 61 houses of the San Diego wood rat, *Neotoma fuscipes macrotis*, from November 4 to December 17, 1943, an average of three bugs per wood rat house. All the insects were taken 5 miles southwest of Fallbrook, San Diego Co., California. Twenty-seven of the 57 bugs naturally infected with *Trypanosoma cruzi* were collected within 150 yards of a large one-story building. The characteristic morphology of stained trypanosomes verified the diagnosis made from inspection of feces for living organisms in the 186 bugs examined. No *Triatoma* were found in the buildings.

The cone-nosed bugs examined included 20 ♂, 31 ♀, 43 large, 25 medium and 67 small nymphs, indicating a tendency for the occurrence in individual wood rat houses of either adults and large nymphs, or small nymphs at that time of year. The 57 (30%) naturally infected bugs included 8 ♂, 9 ♀, 22 large, 11 medium, and 7 small nymphs. The presence of *Trypanosoma cruzi* in the feces of small nymphs would indicate recent infection from the mammal host.

No mammals were trapped, but one ♂ southern parasitic mouse, *Peromyscus californicus insignis*, was caught by hand. Two uninfected bugs, one ♀ and one large nymph, fed on this mouse on November 11, 1943, were negative for trypanosomes when examined 35 days later. Fresh blood examination of the mouse was negative for trypanosomes.

One wood rat house from which 29 *Triatoma protracta* were collected contained 27 infected bugs (3 ♂, 4 ♀, 9 large, 5 medium and 6 small nymphs). The two negative bugs were small nymphs.—Lt. (j. g.) Sherwin F. Wood, H-V(S), U.S.N.R. Forwarding Address: *1015 N. Alexandria, Los Angeles 27, Calif.*

VIABILITY OF *TRYPANOSOMA CRUZI* IN CITRATED BLOOD STORED AT ROOM TEMPERATURE

In the investigation of possible cases of Chagas' disease (American trypanosomiasis) in Texas, blood specimens are collected at various localities and shipped to the central laboratory. In some instances this necessitates a delay of several days before the specimens can be examined. The question arose as to the suitability of such specimens for diagnosis, for it was not known how long *Trypanosoma cruzi* remained viable in citrated, whole blood stored at room temperature.

Material for such a determination consisted of citrated guinea-pig blood parasitized with *T. cruzi*. The animal was inoculated with a culture of the trypanosome on June 9, 1943. Examinations of wet-mount blood preparations on June 14th, 21st, and 26th, revealed no trypanosomes, but on June 28th, nineteen days after inoculation, approximately one trypanosome per low-power field was observed. Cardiac blood was citrated and stored in a sterile, cork-stoppered culture tube at room temperature. Whole blood was tested for *T. cruzi* complement-fixing antibodies by Davis' modification of Kelser's technique; the test was negative in a 1 : 5 serum dilution.

Examinations of the citrated blood consisted of wet-mount and Giemsa-stained preparations, as well as several cultures on Kelser's blood agar slants. Twenty-one examinations were made extending over a period of 257 days.

Cultural forms (leishmania-like forms, crithidia, and metacyclic stages) of the trypanosome were observed in varying numbers in the stored blood over a period of 35 days. On the 43rd day of storage no motile forms were observed in a wet-mount preparation; 0.5 ml of the blood was cultured on Kelser's blood agar slant (27° C). This culture was positive in seven days. Likewise, on the 55th and 144th days of storage no motile forms were observed on direct examination, but positive cultures were obtained. On the 172nd and 257th days of storage motile forms were fairly numerous in the wet-mount preparation. A Giemsa-stained smear of the blood at this time showed that the leishmania-like forms and crithidia were more abundant than the metacyclic stages.

Observations on *T. cruzi* in citrated blood collected early in the infection and stored at room temperature for 257 days, revealed that the trypanosomes not only remained viable but actually multiplied in the blood. It was concluded that citrated blood for the diagnosis of Chagas' disease is satisfactory (by direct examination and culture), even when stored at room temperature, for 257 days, or possibly longer.—THELMA D. SULLIVAN, *Division of Parasitology, Texas State Dept. of Health Laboratories, Austin, Texas.*

NOTES ON ANOPLURA INFESTING MARINE CARNIVORES

From a total of 200 autopsies on seals at the San Diego Zoological Hospital, five incidents of pediculosis have been noted. The hosts include the California sea lion, *Zalophus californianus* Lesson, the Stellar sea lion, *Eumetopias jubata* Schreber and the Harbor seal, *Phoca richardii* Gray.

The lice were not restricted to any particular area on the body of the host; some being found on the head, others on the back and ventral surface. In no case observed was the infestation very heavy. However, all specimens taken were dead at the time of their recovery since the host in each case had unfortunately been placed in refrigeration prior to autopsy.

The lice were identified by the U. S. Dept. of Agriculture as *Antarctophthirus microchir* (Trous.). They belong to the subfamily Antarctophthiriinae, which contains but a single genus *Antarctophthirus* Enderlein (Syn. *Arctophthirus* Mjoberg, 1910).

These lice are large, leathery and spine-studded and are of interest particularly from the standpoint of securing their oxygen supply. Ewing (1929) states, "Perhaps the lice of the family Echinophthiriidae get their air at the same intervals with their hosts when the latter come to the surface of water to breathe."

In the cases cited above, nits were not observed but nymps and larger males and females were collected.—WILLIS H. DOETSCHMAN, *Biological Research Institute, San Diego, Calif.*

THE OCCURRENCE OF *AMBLYOMMA AMERICANUM* IN MINNESOTA AND IN OHIO

To the valuable data of Cooley and Kohls (1944, J. Parasitol. **30**: 77–111) on the distribution of species of Amblyomma in the United States there may be added two previously unpublished records of the occurrence of *A. americanum*, indicating the presence of the species in Minnesota and in Ohio.

On June 4, 1942, I received from a resident of Winona, in southeastern Minnesota a specimen described as "a bug I have found on my person, under my arm. I noticed something there and thought it was a scab, so it remained there for six days." The sender was unable to supply any further significant information. The specimen was a headless ♀, but unquestionably *A. americanum*. No other records for Minnesota are known but there has been no intensive collecting of ticks.

The occurrence of *A. americanum* in southern Ohio would not be surprising in the light of distributional data but apparently it has not been reported for that state. I am obligated to Dr. J. H. Hughes, of the Public Health Service for permission to include in this note the fact that on April 26, 1939, he picked a ♀ specimen from his back, at Jackson, in southern Ohio. He was at the time interested in the ticks of Ohio and recognized the specimen as *A. americanum*. The identification was subsequently verified by Dr. J. C. Bequaert.—William A. Riley, *Division of Entomology and Economic Zoology, University of Minnesota.*

A SIMPLE PERMANENT MOUNTING METHOD FOR *NECATOR AMERICANUS*

Zwemer (1933, Anat. Rec. **57**: 41–44) introduced the method of mounting frozen sections in glychrogel. Wotton (1936, Proc. Staten Island Inst. Arts and Sciences **8**: 8–12 and 1937, Stain Technology **12**: 145–146) has employed the method for mounting trematodes and other small animals. The following glychrogel formula has been used in the preparation of whole mounts of *Necator americanus:*

10%	Bacto Gelatin, granular (Difco)	150 cc.
	Glycerin, reagent (Merck)	50 cc.
1%	Chromium and Potassium Sulfate, C.P. granular (Merck)	100 cc.
	Phenol, U.S.P., liquid	1 cc.

The gelatin is dissolved in boiling water and the glycerin added. After mixing, the chrome alum solution and phenol are added. The medium may gel at room temperature but liquefies in 15 minutes at 65°. Specimens may be transferred from glycerin or formalin solutions to the mounting medium. Slides prepared in this way are ready for examination after 18 hours. The mounting medium hardens into a permanent mount; the specimens remain clear, with mouth parts and genitalia well defined. The method is especially advantageous for use with small nematodes and their ova.—I. Jacques Yetwin, *Middlesex University School of Medicine, Waltham, Mass.*

A HERMIT CRAB AS INTERMEDIATE HOST OF *POLYMORPHUS* (ACANTH.)

Various crustaceans, Amphipoda predominantly, have been reported as intermediate hosts for Acanthocephala, but only two species of Decapoda have thus far been involved—the shrimp *Palaemon squilla,* host of *Arhythmorhynchus roseus,* and the crayfish *Potamobius astacus,* host of *Polymorphus minutus.* In Meyer's table (1933, Bronns Klassen und Ordnungen des Tierreichs., Bd. 4, Abt. 2, p. 340) of known invertebrate hosts for Acanthocephala, the listing of *Calliopius rathkei* under the Decapoda is erroneous since the animal is actually one of the Amphipoda.

During August and September, 1943, at Woods Hole, Mass., I examined more than 8,000 hermit crabs of the species *Pagurus longicarpus* Say and found that in addition to being parasitized by two species of Epicaridea the crabs were also infected with a larval acanthocephalid. The later was identified by John T. Lucker, Bureau of Animal Industry, as a species of the genus Polymorphus Lühe, 1911. Dr. Harley J. Van Cleave, to whom the specimens were later submitted, confirmed the generic identification and stated that a specific determination could not be made with reasonable certainty unless mature worms could be secured.

Polymorphus occurs in encysted condition in the abdominal cavity of the hermit crab. Usually the cysts are firmly attached to the outer wall of the mid- or hind-gut, but occasionally they occur among the tubules of the hepato-pancreas. One cyst per host is the usual condition but two or three were sometimes found in the same crab. Approximately one per cent of the hermit crabs from Great Harbor were infected with this acanthocephalid, but no case of simultaneous parasitism involving both Epicaridea and Acanthocephala was noted.

In shape the cysts of *Polymorphus* are oval or fusiform. They average 2.59 mm. in length (maximum 3.12, minimum 2.16) and 1.27 mm. breadth (maximum 1.44 minimum 1.20). Because of their relatively large size and glistening whiteness their presence can be detected without dissection of the crab. The body wall of the host's abdomen is transparent enough to reveal the cysts when the living crabs are examined under a low-power binocular dissecting microscope.—Edward G. Reinhard, *Catholic University of America, Washington, D. C.*

GIARDIA SANGUINIS (GONDER, 1911) NOT FROM A "FALCON"

Hegner and Taliaferro (1925, Human Protozoölogy) and Wenyon (1926, Protozoölogy) state that Gonder (1911, Arch. f. Protist. **21**) described a *Giardia* from the blood of a "falcon." This declaration has been perpetuated in a number of references, the most recent of which is by Herman (1943, J. Parasit. **29**). There is here a slight misimplication, however, which may be worth correcting.

Gonder's *Giardia* came from "ein blauer Falke, *Elanus coeruleus.*" Now "falke" may mean falcon, hawk or kite, but *E. coeruleus* is neither falcon nor hawk. It is a kite (Accipitridae), and taxonomically is not even in the same family with the falcons (Falconidae). As continued reference to this host as a falcon leads to confusion ornithologically, it seems more appropriate to call Gonder's bird what it was, a kite.—Robert M. Stabler, *Department of Zoölogy, University of Pennsylvania, Philadelphia, Pa.*

XENOPSYLLA CHEOPIS IN LINCOLN, NEBRASKA

Two fleas (♀ and ♂) taken from an immature house rat, *Rattus norvegicus* (Erxleben), at Lincoln, Nebraska, December 28, 1943, have been identified as *Xenopsylla cheopis* Roths. The rat was collected in the basement of a home in Lincoln. Several specimens confirmed as *X. cheopis* were collected from a rat in Lincoln about eight years ago by parasitology students at the University of Nebraksa, but exact data on this collection are not available. These records indicate that probably the species has become established in this area. Further efforts will be made, as circumstances permit, to determine the extent to which it occurs here.

It is not surprising to find *X. cheopis* in this area since it was reported from Ames, Iowa, in 1934, and Manhattan, Kansas, in 1941.—Doris B. Gates, *Department of Entomology, Nebraska Agricultural Experiment Station, Lincoln, Neb.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirty-Second Council Meeting, Baltimore, Maryland

January 29, 1944

The meeting of the Council of the American Society of Parasitologists was called to order by Dr. Henry E. Meleney, President of the Society, at 2:00 PM, January 29, 1944, in the Department of Parasitology, Johns Hopkins University, 615 N. Wolfe Street, Baltimore, Maryland. Past-president W. W. Cort and the following members of the Council were present: D. L. Augustine, E. R. Becker, H. E. Meleney, G. F. Otto, E. W. Price, L. E. Rozeboom, H. W. Stunkard, and W. H. Wright.

I. Reports of Officers

1. *Secretary* (*J. T. Culbertson*): As of December 31, 1943, there were 520 persons on the membership roll of the Society, of whom 454 lived within and 66 lived outside continental United States. Of those on the roll, 419 were members in good standing and 101 were delinquent in dues for either 1 or 2 years. Of those in good standing, 380 lived within and 39 lived outside continental United States. Twenty-eight persons were elected to membership during the year, of whom 25 lived within and 3 lived outside continental United States. One member died during the year: Dr. William A. Hoffman, Assistant Professor of Parasitology, Columbia University, School of Tropical Medicine, San Juan, Puerto Rico.

Upon motion, the Secretary's report was accepted and placed on file.

2. *Treasurer* (*L. E. Rozeboom*): For the fiscal year 1943 (Dec. 7, 1942 to Dec. 6, 1943):

Receipts

Balance on hand, Dec. 7, 1942 (J. Parasitol. **29**: 236)		$2525.15	
Collected during current year			
Member dues applying 1943	$ 756.51		
Member dues applying 1944	440.00		
Advance dues	8.00	1204.51	
Subscriptions applying 1943	1719.55		
Subscriptions applying 1944	377.00		
Advance subscriptions	11.25	2107.80	
Back vols. and nos. sold	189.25		
Arrearages	52.00		
25-vol. index sales	59.40		
Contributions from members	89.00		
Advertisements	190.00		
Author charges	91.00		
Miscellaneous	27.87	698.52	
Grant from Amer. Found. Trop. Med.		1000.00	
			$7535.98

Expenditures

Printing journal			
1942 account	$ 829.04		
1943 account	2698.15		
		$3527.19	
Expenses in office of Chm. Ed. Comm.		147.44	
Expenses in office of Secretary		102.77	
Expenses in office of Treasurer		324.52	
Payment of loan from Princeton Fund		402.00	
Miscellaneous		74.90	
			4578.82
Balance on hand, Dec. 6, 1943			$2957.16

Upon motion, the Treasurer's report was accepted, subject to audit, and placed on file.

3. *President (H. E. Meleney)*: Correspondence with Dr. H. B. Ward with respect to back numbers of the JOURNAL OF PARASITOLOGY had revealed that the number of copies of early volumes now in Dr. Ward's possession was not large enough to justify his transferring them to the Society.

During the last several weeks, the members of the Society were canvassed to determine their participation in war activities. The returns are now being studied and an analysis of the findings will be published in the JOURNAL OF PARASITOLOGY in the near future.

II. REPORTS OF COMMITTEES

1. *Custodian of the Princeton Secretarial Fund (N. R. Stoll)*:

	Dec. 3, 1943	*One year ago*
A. Total liquid assets	$998.55	$ 475.79
B. Book value subsidiary assets	None	816.67
Total	998.55	1292.46

Upon motion, the report was accepted, subject to audit, and placed on file. A vote of thanks was extended to the Custodian for his careful supervision of the Fund.

2. *Chairman of the Editorial Committee (N. R. Stoll)*: During 1943, the six regular numbers of volume 29 of the JOURNAL OF PARASITOLOGY containing 444 pages (equivalent to 555 pages of the earlier format) were published. Sixty-five regular articles and 28 research notes were included in the volume. The interval between "received for publication" and date published was, for the regular articles, 9.2 months (compared with 11.0 months in 1942) and, for research notes, 8.6 months (compared with 10.3 months in 1942). The year was marked by a continuation of the trend, noted earlier, of a falling off in submitted manuscripts. The entrance of many members of the Society into the armed forces was considered the chief reason for fewer manuscripts being received.

During 1944, by order of the War Production Board, only 90 per cent, by weight, of the paper used in 1942 can be obtained for printing the JOURNAL. Fortunately, because of the new format, this reduction in available paper will not reduce the amount of material published.

During the year, with approval of the Editorial Committee and Board, and of Council, application was made to the American Foundation for Tropical Medicine for a subvention of $1,000 to aid in publishing the JOURNAL OF PARASITOLOGY, on condition "that the Foundation would grant it without restriction, use of the money to be at the discretion of the Committee, present standards of the JOURNAL OF PARASITOLOGY to be upheld." The Foundation promptly granted the sum, thus permitting the Editorial Committee to relax its charge on authors when this was less than $5.00, as well as its 10-page limit on the size of articles. Actually, the Foundation grant was unused at year-end and was turned over intact to the new Editorial Committee.

During 1943 there was a total of 822 paid members and subscribers, 3 less than in 1942. Especially heartening as a sign of loyalty to the JOURNAL was the voluntary contribution of $313.00 by 145 dues-paying members during the year.

During the year, there were 31 exchanges on the JOURNAL list. This number could easily be doubled, at least when the war ends, without creating a serious financial burden.

Thirty copies of the 25-year Index were sold in 1943, bringing the total number sold to 400 of the 1200 printed. Consideration is invited of the judicious distribution of the Index to libraries in South and Central America and after the war ends to devastated libraries in war areas, perhaps through the American Library Association Committee.

Sets of volume 29 of the JOURNAL were purchased by the American Library Association Committee for devastated libraries in war areas.

Upon motion the report was accepted and placed on file. A vote of thanks was extended to Dr. Stoll and the Editorial Committee for its services, as well as to the Rockefeller Institute at Princeton for supplying clerical service afforded to Dr. Stoll while Chairman of the Editorial Committee.

3. *Committee to Audit Reports of Treasurer and Custodian of Princeton Secretarial Fund (G. F. Otto, W. W. Cort)*: The Auditing Committee approved the reports of the Treasurer and of the Custodian of the Princeton Secretarial Fund.

Upon motion, the Committee's report was accepted and placed on file.

III. NEW BUSINESS

1. *New Members:* Council voted membership in the Society upon two applicants: Dallas K. Meyer, Aquatic Biologist, United States Fish and Wildlife Service, Ft. Worth, Texas, and William Cleland Forbes, King's College, New Castle, Delaware.

2. *Management of the Journal:* Council voted to give the Editorial Committee power to decide its own policy for increasing the number of submitted manuscripts or the size limit of acceptable manuscripts.

3. *Selection of Place for the 1944 Meeting of the Society:* No decision was reached as to time or place of meeting in 1944.

4. *Election of Officers, Appointments, etc.*

a. Council voted to confirm the mail vote by members of the Society which was taken late in 1943. Accordingly, the following were elected to the Society's offices: Henry E. Ewing, *President* for one year; Benjamin Schwartz, *Vice-President* for one year; L. E. Rozeboom, *Treasurer* for one year; J. T. Culbertson, *Secretary* for two years; H. W. Manter and E. W. Price, *Councillors-at-Large* for three years; and C. G. Huff and H. W. Brown, *Councillors-at-Large* for four years.

b. The following persons were elected by Council to the Editorial Board of the JOURNAL OF PARASITOLOGY: L. T. Coggeshall, J. T. Lucker, and N. R. Stoll.

c. *Committee on Terminology of Strains of Avian Malaria:* Council voted to continue the Committee (C. G. Huff (Chairman), G. H. Boyd, and R. D. Manwell) for one year and recommended that a supplement to the last report of its activities be published.

d. *Committee on Nomenclature:* The present Committee, consisting of E. W. Price (Chairman), B. G. Chitwood, and A. McIntosh, was reappointed, with power to represent the Society on the General Council on Zoological Nomenclature.

e. *Committee on Common Names of Parasites:* A committee to decide on acceptable common names of parasites of man and other animals was erected. P. D. Harwood was named Chairman and two other members were to be selected later.

f. *Representatives on the Council of the American Association for the Advancement of Science:* A. C. Chandler and H. J. Van Cleave were named as representatives for one year.

g. *Representatives on the Council of the Union of American Biological Societies:* A. O. Foster and A. C. Walton were named as representatives for one year.

h. *Inactive or Suspended Membership:* Council voted that members of the Society who, because of the war, are outside continental United States or temporarily out of touch with their usual parasitological activity are to be given inactive or suspended status if they become delinquent in payment of dues. They are not to be dropped from the Society membership roll for the duration of the war. They can resume active membership at any time on payment of current dues, and on payment of delinquent dues may receive back numbers of the JOURNAL.

Council voted its thanks to the Society Officers and Representatives and adjourned.

Respectfully submitted,

JAMES T. CULBERTSON, *Secretary.*

Second Report of the Committee on Terminology of Strains of Avian Malaria

The proposal made by this committee in the supplement to the report of the 17th annual meeting of the American Society of Parasitologists (J. Parasitol. **28**: 250, 1942) that a uniform terminology of strains of avian malaria be adopted has met with uniformly favorable reactions on the part of investigators. The designations of strains and substrains made then have been widely used. The committee holds the opinion that this practice is aiding in preventing confusion in the terminology applied to strains of avian malaria and recommends its continuance. Designations made subsequent to the previous report are listed in tables 1 and 2. For an explanation of the principles guiding the committee in applying the designations reference is made to the previous report, Jour. Parasitol. **28**: 250–254, June, 1942.

Again it is urged that investigators use the official designations of strains when publishing the results of their experiments or when sending the strain to others. The committee will be pleased to make designations of new strains or substrains prior to the publication of experimental work upon such strains. We wish to express our appreciation for the cooperation shown already on the part of all investigators involved. We are also pleased to extend our service to scientists in other countries.

COMMITTEE ON TERMINOLOGY OF STRAINS OF AVIAN MALARIA

Clay G. Huff, *Chairman*
George H. Boyd
Reginald D. Manwell

TABLE 1.—*Designations of original strains*

Designation	Host source	Host locality	Isolated by	Date of isolation	Other names used	Reference
P. relictum 1 C	*Excalfactoria lineata* (Island painted quail)	Philippine Is.	P. F. Russell	1932	*P. capistrani*	Phil. J. Sci. **48**: 269 (1932). (Designation requested by Dr. F. Wolfson. Amer. J. Trop. Med. **16:** 685 (1936))
P. cathemerium 3 C	*Passer domesticus*	Oshkosh, Wis.	P. E. Thompson	1944		None
P. elongatum 5 C	*Sialis s. sialis* (Bluebird)	Atlanta, Ga.	W. B. Redmond	1942		None
P. elongatum 5 D	*Passer domesticus*	Indianapolis, Ind.	E. Price	1943		None
5 M	*Sturnella magna* (Meadowlark)	Kansas, Ill.	C. G. Huff	1934	ML	J. Inf. Dis. **57**: 315 (1935)
P. circumflexum 6 F	*Passer domesticus*	Oshkosh, Wis.	P. E. Thompson	1944		None
P. juxtanucleare 14 A	*Gallus domesticus*	Western part of Minas Geraes, Brazil	Versiani and Gomes	1941		Rev. Brasil. Biol. **1**: 231 (1941)
14 B	*Gallus domesticus*	Huixtla, Chiapas, Mexico	E. Beltran	1942		Rev. Inst. Salub. y. Enfr. trop. **2**: 353 (1941)

TABLE 2.—*Designations of sub-strains*

Designation	Source	Derivation and investigator	Characteristic	Reference
1P1 (*P. relictum*)	1P, Coatney's pigeon strain	W. B. Redmond	Adapted to blood infection in canary. Infects *Culex* mosquitoes	Bull. Ga. Acad. Sci. **1**: 9 (1943). J. Inf. Dis. **74**: 184–188
1P1–1 (*P. relictum*)	1P1	Through mosquito passage by W. B. Redmond	No longer infects pigeon either by inoculation of blood or sporozoites or by bite of infected mosquito	Bull. Ga. Acad. Sci. **1**: 9 (1943). J. Inf. Dis. **74**: 184–188
3H1–3 (*P. cathemerium*)	3H1–2 or "P" strain	Treated in vitro with high sublethal temperatures by F. E. Caldwell	Shows marked disturbance in synchronism of schizogony	J. Inf. Dis. **74**: 189–205
3H1–4 (*P. cathemerium*)	3H1–2 or "P" strain	Treated in vitro with high sublethal temperatures by F. E. Caldwell	Shows marked disturbance in synchronism of schizogony and loss of gametocytes. No longer capable of transmission by mosquitoes	J. Inf. Dis. **74**: 189–205
3H1–5 (*P. cathemerium*)	3H1–2 or "P" strain	Treated in vitro with high sublethal temperatures by F. E. Caldwell	Not noticeably changed from parent strain	J. Inf. Dis. **74**: 189–205
12A1 (*P. lophurae*)	12A	Adapted to canary by F. Coulston	Capable of producing heavy infections in canaries	None